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THE THYMUS IN RELATION TO MYASTHENIA GRAVIS

DONALD McEACHERN, M.D.[1]

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1. INTRODUCTION

The general character of myasthenia gravis suggests an endocrine or metabolic disorder. Onset is usually after puberty and in early adult life; there is a tendency to remissions. Modifications of the disease may occur during pregnancy or with changes of thyroid function. At autopsy no structural change is found in the nerves or muscles except for the presence of scattered lymphorrhages, which can scarcely be considered causative. The only other common finding is hyperplasia or tumor formation in the thymus, which occurs in about half the cases.

The clinical pictures associated with hyperfunction of various known endocrine glands are quite clear and often point to the normal action of those glands. Many examples could be cited: islet tumor of the pancreas and hyperinsulinism, parathyroid adenoma and fibrocystic bone disease, adrenalin-forming pheochromocytoma and paroxysmal hypertension. Although these conditions may be rare, they nevertheless vividly illustrate the normal functions of the glands in-

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volved. It is apparent that the high incidence of abnormality of the thymus in myasthenia gravis might have similar significance. Further studies of this relationship are in order.

2. POST MORTEM EVIDENCE OF THYMIC ABNORMALITY

A. Incidence of Abnormality in Myasthenia Gravis

Norris (61), in 1936, collected the reports of cases of myasthenia gravis which had been autopsied up to that time. Thirty-five out of the total of 80 cases showed lesions of the thymus. In the same year Lievre (52) made a somewhat different tabulation of 67 cases on which complete autopsies had been performed. Thymic tumor was said to be present in 24, and persistence or hypertrophy of the thymus in 32 cases. No abnormality was found in the remaining 11. Blalock et al. (8) have completed the tabulation down to 1939. In 110 operations or autopsies on patients with myasthenia gravis some abnormality of the thymus occurred in 53 instances. Thirty-one of these were classified as tumors, 22 as enlargement or persistence of the thymus.

It is apparent that the tendency to report only cases in which thymic abnormality has been found may make the incidence of such anomalies higher than is actually the case. This is counterbalanced by the ease with which small or misplaced thymic tumors can be missed unless very special search is made. The mediastinum is a difficult region for such a search. Indeed, small but possibly potent nodules could only be excluded with certainty by making serial microscopic sections of the entire region. Foster Kennedy (43) studied 8 consecutive cases at autopsy. No frank thymic tumors were found, but in 5 cases there was a paper-thin sheet of thymus tissue covering the pericardium. This important observation requires confirmation.

B. Type of Abnormality in Myasthenia Gravis

The classification of thymic tumors is in a muddled state, and the terms "thymoma" and "lymphosarcoma" have covered a multitude of sins. It is sufficient for our purpose to note that the large majority of tumors associated with myasthenia gravis have been nonmalignant, encapsulated nodules of small size and soft, hemorrhagic character. The average tumor weight in Lievre's (52) series was 60 gms., average diameter 5 cm. Blalock et al. (8) considered that only one instance had been recorded in which myasthenia was associated with a malignant tumor. Of 8 autopsies performed at the Mayo Clinic (21), thymus abnormality was found in 2 instances. One case showed hyperplasia, the other a malignant tumor.

When frank tumor formation is lacking there may be diffuse enlargement and hyperplasia of the thymus. Norris (61) felt that thymic anomalies in myasthenia gravis could best be interpreted as greater or lesser degrees of epithelial hyperplasia. "When the hyperplasia is extreme, a localized, and at times encapsulated, tumor-like mass is formed." There is some doubt as to whether the hyperplasia involves epithelial elements, lymphocytes, or both.

C. Other Conditions Associated with Thymic Hyperplasia

The existence of status thymico-lymphaticus is still a matter of debate. The present fashion is to deny its existence, but some experienced clinicians and pathologists still cling to it as a possibility. It seems clear that past attempts to explain sudden death in such cases on the basis of mechanical respiratory obstruction by an enlarged thymus have failed. The possibility of an endocrinological explanation has not been considered. If the thymus of an affected individual suddenly released an excess of a curare-like substance, under conditions of struggle, stress or the inhalation of an anaesthetic, there would be a ready explanation for death by sudden respiratory failure or laryngeal spasm.

Two points are of interest in this regard. Coe (17) has described a number of sudden deaths in infants submitted to minor operations. He noted that death resulted from respiratory paralysis whilst heart sounds continued for some time. There was no evidence of respiratory obstruction. He postulated the release of some curare-like substance. Myasthenia gravis is seldom seen before puberty. It is possible, however, that the underlying defect may be present in children but that the clinical picture may be different from that seen in adults; thus, respiratory muscles might be primarily affected rather than those innervated from the brain stem. Two of our patients who developed myasthenia gravis at the time of puberty had previously had periodic respiratory symptoms for some years. One suffered from frequent smothering spells at night, the other from "asthmatic" attacks which, on later questioning, were found to be brought on only by effort, running, wrestling, etc., and which passed off after 10 to 15 minutes rest.

Another suggestive association is to be found in 3 endocrine disorders: hyperthyroidism, adrenal cortex deficiency and castration, each of which has in common the triad: muscular asthenia, creatinuria and hypertrophy of the thymus.

3. NATURE OF NEUROMUSCULAR DISTURBANCE IN MYASTHENIA GRAVIS

Myasthenia gravis is characterized by undue fatigability and, in some severe cases, by some persistent weakness of the skeletal muscles. Function of the heart and visceral muscles is unaffected. As a rule the ocular muscles and those innervated from the bulb are first and most severely involved. Later the limb muscles may become affected, and finally the respiratory muscles. Death may result from respiratory failure or from sudden spasm of the glottis. Two of our patients died with laryngeal spasm, one of them having developed sudden respiratory obstruction while in a Drinker respirator which was functioning normally. Autopsy revealed that the air passages were quite clear.

There is an unmistakable resemblance between the above phenomena and the more fleeting symptoms produced by intravenous injection of curare or its derivatives in the human. These have been well described by West (86), Bennett (7) and Harvey and Masland (33). Small doses cause ptosis of the eyelids and weakness of the extraocular muscles, followed shortly by weakness of the muscles of mastication, deglutition, articulation and phonation. With larger doses limb

weakness appears, and finally weakness of the respiratory muscles which, according to West (86), may be punctuated at any moment by sudden spasm of the glottis.

It has long been known that the main defect in both myasthenia gravis and curare poisoning is of the nature of a block or barrier to motor nerve impulses either at the myo-neural junction or in the muscle fibre. Under the influence of curare, conduction within the nerve is normal and the muscle responds to direct stimulation, but stimulation of the nerve causes rapid loss of muscular contraction or none at all. In mildly curarized animals Briscoe (13) has described a myographic abnormality of similar significance and akin to Wedensky inhibition. When the motor nerve is stimulated at low frequencies (30–60 per sec.) the muscle responds with normal, sustained tetanus. At high frequencies (over 100 per sec.) the muscle responds, but only momentarily in the form of a twitch. Normal tetanus returns after appropriate doses of prostigmine. With increasing doses of curare there comes a time when no additional amount of prostigmine will correct the abnormality. Indeed, prostigmine alone in large dosage can produce a somewhat similar abnormality of neuromuscular conduction due to paralyzing concentrations of acetyl choline at the motor nerve endings. There is a parallel between the above findings and clinical experience with prostigmine. The patient with mild myasthenia may sometimes be restored to a temporarily normal state with an appropriate dose of the drug, whereas in some severe cases no amount of the drug will carry recovery beyond a certain point; indeed, a smaller dose may prove more effective than a larger one.

The above myographic phenomena may also be observed in myasthenia gravis, as first pointed out by Pritchard (66). The well-known Jolly test is probably an example, since faradic (high frequency) stimulation causes rapid fatigue, whilst galvanic (single shock) stimulation can be repeated indefinitely without fatigue. Pritchard's technique for humans is similar in principle to that used by Briscoe (13) for animals. Stimulation of the ulnar nerve is carried out at any desired frequency and the myogram is recorded from the little finger. Normal individuals respond with sustained tetanus at any frequency. Patients with myasthenia gravis show normal tetanus at low frequencies (below 100 per sec.), a twitch response at high frequencies (above 100 per sec.). Russel, Odom and McEachern (70) have investigated this response in a series of cases of neuromuscular diseases. Wedensky inhibition was found in all cases of myasthenia gravis and in 2 cases of chronic thyrotoxic myopathy with marked muscular fatigability, but was absent in patients with other muscular or neuromuscular diseases. It was improved or abolished by prostigmine. Intra-arterial injection of prostigmine abolished the defect in the injected limb within a few seconds. In the opposite limb the defect persisted until about five minutes after a venous tourniquet was released from the injected limb and the prostigmine thus allowed to enter the general circulation. The effect of prostigmine is therefore peripheral. Coincident studies of blood cholinesterase showed a reduction which corresponded with the muscular improvement due to prostigmine. After intra-

rial injection, however, the myogram from the injected limb remained nal for some time after the circulating blood cholinesterase had risen to the injection level. This would indicate that persisting improvement was due ›duction of the muscle cholinesterase in the injected limb despite the level of ılating cholinesterase. The myographic defect in myasthenia gravis is efore essentially similar to that found in curarized animals. Similar con-ons can be drawn from the studies of Harvey and Masland (34) and Harvey, nthal and Talbot (31) on humans with myasthenia gravis and persons under influence of various curarizing drugs.

he newer knowledge of chemical mediation of neuromuscular transmission naturally led to attempts to relate myasthenia gravis to an aberration of ıoral transmission. Our ideas of normal mediation can be illustrated by ıre 1.

n stimulation of the motor nerve fibre, acetyl choline is exploded in minute unt at the myoneural junction or in immediate proximity to the surface of

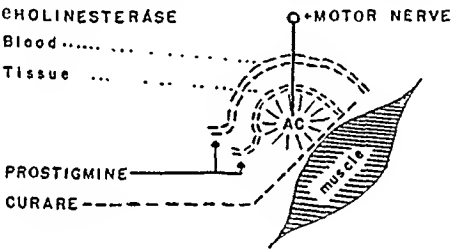


FIG. 1

muscle fibre. This either causes muscular contraction or so conditions the e that it responds normally to the electrical impulse. The enzyme, chol-terase, present both in the muscle tissue and in the circulating blood, rapidly ıks down any excess acetyl choline, thus preventing its spread to adjacent ·cle fibres or into the general circulation, and limiting its effect to the desired ıt of stimulation. Eserine, or prostigmine, has an affinity for cholinesterase, ls it and takes it "out of the play", thus allowing a greater concentration cetyl choline to exert its effects. These anti-cholinesterase drugs, therefore, entiate and prolong the muscular stimulation by acetyl choline.

'urare and similar drugs have no effect upon the production of acetyl choline ; they may not alter the activity of cholinesterase, but in some way they uce the response of the motor end plate or the muscle fibre to nerve stimula-ı (Dale et al. (20)). If curarization is not too heavy prostigmine will over-e the defect by mobilizing more acetyl choline. There is a limit to this, ıever, when all the cholinesterase has been bound and all the available acetyl line is in action. A larger dose of curare can then not be surmounted by · dose of prostigmine.

Events might be schematically pictured as in figure 2, although this may be too facile an explanation:

1. In the normal resting nerve-muscle preparation, acetyl choline is held in the reservoir and the muscle remains quiescent.

2. Upon stimulation of the nerve in the normal preparation, acetyl choline is released from the reservoir and causes muscular contraction.

3. In the moderately curarized preparation, a normal amount of acetyl choline is released but the curare acts as a partial barrier and the full amount of acetyl choline does not reach the muscle.

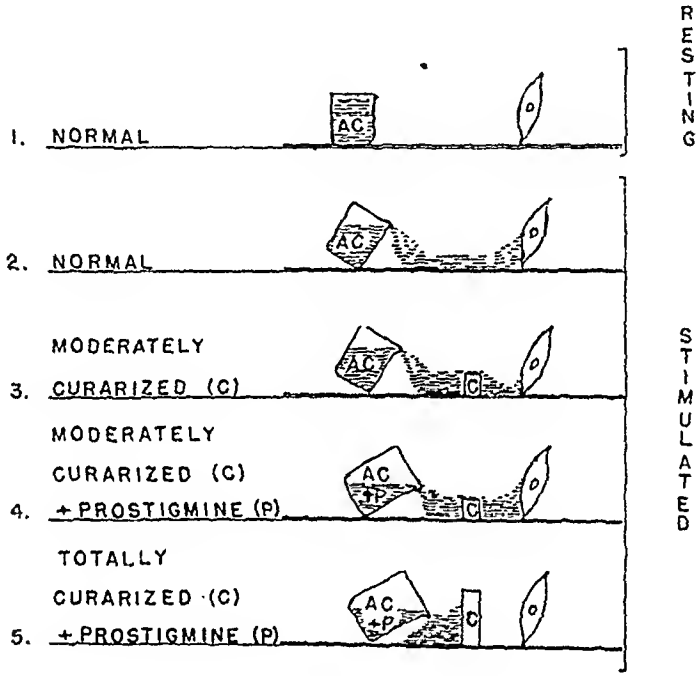


FIG. 2

4. The effect of prostigmine permits the mobilization of a supernormal quantity of acetyl choline and the effect is sufficient to cause normal muscle stimulation despite the curare barrier.

5. The curare barrier is so high that despite mobilization of a super-normal amount of acetyl choline, not enough of the substance reaches the muscle to cause contraction.

In this schema the curare could be looked upon as forming a surface film on the reacting part of the muscle rather than acting as a mechanical barrier as pictured.

It was at first thought that the myasthenic patient might have too much circulating cholinesterase, and that his acetyl choline might be continually destroyed at too rapid a rate. This seemed unlikely on clinical grounds, since a wide variety of visceral and autonomic functions which are dependent on cholinergic transmission (heart, stomach, bladder, etc.) should have been, but are

not affected. Direct measurements by Stedman and Russel (74), McGeorge (53), Milhorat (55) and Russel, Odom and McEachern (70) have proved, moreover, that no increase of cholinesterase is present in the blood in myasthenia gravis. McGeorge was unable to demonstrate any anti-cholinesterase activity in the urine of 2 patients with myasthenia gravis. Two hours after prostigmine, however, urine showed marked depressant effect on the esterase, thus suggesting excretion of the drug in the urine. There remains the possibility that cholinesterase might be increased at the motor nerve endings or in the muscle. The only two observations on this point have been made by Jones and Stadie (42) and Goodman, Carlson and Gilman (25). No abnormality was found.

It has been suggested, too, that the myasthenic patient might suffer a lack or deficiency of acetyl choline at the motor end plates. This is hard to prove or disprove. Again, a generalized deficiency of acetyl choline or its precursors might be expected to cause dysfunction of visceral organs which are just as dependent upon cholinergic transmission as are the skeletal muscles. Such is not the case. It is conceivable, however, that acetyl choline might be deficient at the motor end plates and not at other cholinergic nerve endings. Fraser et al. (22) claimed benefits in myasthenia from the injection of the more stable choline esters mecholyl and doryl. If true, this would point to a deficiency of acetyl choline or its precursors at the motor end plates. These results have not been confirmed by others (47, 63) or by our own experience. No direct measurements have been made of production or synthesis of acetyl choline in myasthenic muscle.

There remains the possibility that some as yet unidentified curare-like substance is responsible for the condition. This explanation would best accommodate the facts known at present. Proof, however, awaits the demonstration of such a substance. The few experiments that have been aimed in that direction will be described later.

Lanari (50) first showed that the muscles in myasthenia gravis are extraordinarily sensitive to acetyl choline when it is injected intra-arterially. This is quite unlike the response of partially curarized muscle, which is less sensitive than normal. Harvey and Lilienthal (32) consider this as evidence that a deficient amount of acetyl choline is released at motor nerve endings in myasthenia gravis. They suggest that circulation of a curare-like inhibitor substance may be the primary fault. It would be interesting to know whether curarization extending over several weeks would sensitize muscle to acetyl choline injected intra-arterially.

Nowotny and Redlich (63) claim that the tissues of myasthenic patients show a diminished capacity to utilize oxygen from the arterial blood. Prostigmine is said to correct this disturbance of cellular metabolism. The authors' figures are not consistent, however, and their claim requires confirmation.

Much has been made of Kennedy and Wolf's (45) suggestion that myasthenia gravis and myotonia are clinical opposites. This view is held because the former condition is characterized by undue muscular fatigability, the latter by too pro-

longed contraction, and also because of opposite responses to the following drugs:

	MYASTHENIA	MYOTONIA
Anticholinesterase drugs,* e.g., prostigmine.....	Improve	Make worse
Curariform drugs, e.g., quinine.....	Make worse	Improve

* It should be mentioned that the anticurare power of a drug does not necessarily parallel its anticholinesterase properties. Indeed, quinine, which has a curariform action is capable of lowering the cholinesterase, as was shown by Jacobsohn and Kahlson (41).

This explanation may be too facile. Certainly prostigmine in large doses does not produce myotonia in the animal or human, but instead, generalized muscular fasciculations and cramps. Larger doses, according to Briscoe (13), produce interference with normal muscular contraction but not failure of normal relaxation as is seen in myotonia. Kennedy and Wolf (45) transfused 500 cc of blood from a patient with myotonia congenita to one with myasthenia gravis. No effect was noted.

4. EXPERIMENTAL WORK ON THE THYMUS

A. Attempts to Demonstrate Endocrine Function

A vast amount of experimental work has failed as yet to prove any secretory function for the thymus. The evidence has been well considered in the monumental paper of Park and McClure (65) and the more recent reviews by Crotti (19), Gudernatsch (28) and Nelson (58).

The classical methods of endocrine research designed to produce deficiency symptoms upon removal, and evidences of positive effect on administration or surgical implantation of a gland, have been negative with the thymus. The results of Gudernatsch (27) showing increased growth and early differentiation in tadpoles and increased growth of rats due to feeding of thymus, and the increased growth rate of body and gonads of rats treated with Asher's (5) thymic extract "thymocresin" do not seem to be disputed, but it is not necessary to consider the results as evidence of an endocrine function for the thymus. The more recent results of Rowntree (69) and his collaborators, pointing to precocious development in succeeding generations of rats injected with a thymic extract (Hanson), have not been confirmed by other workers (Segaloff and Nelson (72)).

Several recent but unconfirmed studies should be mentioned. Adler (2) claimed to have reproduced the symptoms of myasthenia gravis in dogs by the implantation of thymic tissue or the injection of extracts, and to have abolished symptoms with prostigmine. Our own experiments will be discussed later. Those that resemble Adler's do not confirm his results. Adler (3) also felt that the muscular asthenia associated with hyperthyroidism and with adrenal cortex deficiency is due to coincident hyper-activity of the thymus. This alluring possibility has occurred to many of us, but is without proof. Bomskov and Milzner (10), using rats and guinea pigs, could not confirm Adler's results with dogs.

Bomskov and Sladovic (11) claim to have identified a "thymotropic hormone"

of the anterior pituitary, and also the "thymus hormone." The thymus hormone is extracted with fat solvents whilst the thymotropic hormone is a protein body which is said to be identical with the "diabetogenic hormone" = growth hormone of the anterior pituitary. In animals, the thymus hormone produces fall of liver glycogen, rise of blood sugar, lymphocytosis and glycosuria. The final action of both hormones is to mobilize carbohydrate. According to Hoepke (36), the thymus hormone is carried by lymphocytes and not in the fluid elements of the blood. Wide speculations are made as to the normal functions of both hormones. Wells, Riddle and Marvin (85) failed to confirm Bomskov's findings with thymic extracts.

Sunder-Plassman (75), in an interesting anatomical study, stated that when the thyroid gland is "activated" by pre-treatment of the animal with thyrotropic hormone or "foreign proteins", large "neuro-humoral" cells appear in the thyroid in relation to vagus nerve endings. These appear to be none other than large thymic epithelial cells which have emigrated from that organ to the thyroid via perivasal nerve plexuses or the neuro-reticulum of the pre-tracheal fatty tissue. Sunder-Plassman followed this migration with serial sections. He considered that thymus and thyroid are bound through the parasympathetic nervous system into a biological complex with a particular function. The significance of this finding is not clear, but it is of interest in view of the known relationship of thymus bulk to thyroid activity.

B. Hypertrophy and Involution of Thymus Due to Changes in Function of Certain Endocrine Glands

It has been shown that the thymus undergoes hypertrophy or involution with changes affecting some endocrine glands. This evidence, both clinical and experimental, is suggestive of an endocrine function for the thymus and can be assembled as follows:

CONDITION	THYMUS	AUTHOR
Hyperthyroidism	Hypertrophy	Reinhardt & Wainman (68) Selye (73) Crotti (19)
Hypothyroidism	Involution	Reinhardt & Wainman (68) Selye (73) Crotti (19)
Adrenal cortex hyperfunction	Involution	Houssay (37) Ingle (40) Moon (57) Selye (73)
Adrenal cortex hypofunction	Hypertrophy	Reinhardt & Holmes (67) Houssay et al. (38) Selye (73) Tobin (78)
Sex gland hyperfunction (male and female)	Involution	Reinhardt & Wainman (68) Chiodi (16) Anderson (4) Nelson (58)
Sex gland hypofunction (male and female)	Hypertrophy	Reinhardt & Wainman (68) Chiodi (16) Anderson (4) Nelson (58)

Noble and Collip (60) showed that saline suspensions of whole pituitary, given along with an adrenal-corticotropic extract, may produce marked hypertrophy of the atrophied thymus in hypophysectomized rats. Although the corticotropic extract alone might be expected to cause thymic atrophy this was reversed when the augmenting extract was administered. The latter extract, weak in corticotropic and gonadotropic action, had definite growth-promoting properties, and when administered alone was strongly thymotropic.

The effects of hypophyseal gonadotropic and adrenocorticotropic hormones on the thymus are mediated through the gonads and the adrenal cortex respectively and do not appear if the latter glands have been previously removed. Pregnancy is said to cause involution of the maternal thymus, as first noted in cattle by Henderson (35).

5. EVIDENCE FOR POSSIBLE THYMUS HORMONE

Since it is the fashion nowadays to deny the possibility of any endocrine function of the thymus (see Kingsbury (46)), an attempt will be made to assemble some of the suggestive but inconclusive evidence to the contrary.

A. Origin and Development of the Thymus

The development of the human thymus has been beautifully studied by Norris (62). The organ is derived from the third branchial complexes. The parathyroids develop from these complexes, and the thyroid largely from the fourth. The human thymus first makes its appearance as two endodermal outbuddings of the third branchial complex in the 9 to 12 mm. stage of embryonic life, and by the third month it is well developed, the two lobes lying side by side and bound together in the anterior mediastinum anterior to the pericardium. Norris has shown that the thymus of man is an ectodermal-endodermal structure. The ectoderm of the cervical sinus is the primordium of the primitive thymus cortex and the source of the Hassall's corpuscles. The syncytial cytoreticulum of the organ is derived from the endoderm of the third branchial pouches. The fibrous reticulum is derived from connective tissue of the capsule and of the adventitia of blood vessels. The lymphocytes are of mesenchymal origin and invade the thymus from without.

B. Weight of Thymus in Relation to Age and Body Weight

The normal weight of the organ at various ages is shown in the table on page 11 taken from Crotti (19).

The figures taken from Hammar's extensive investigations are the most reliable. He considered as normal only the thymuses of persons who died of accidental causes within from 24 to 48 hours of injury. By 1929 his experience was based on a study of about 800 thymuses (30). It is well known now that "normal" thymic weights based on organs removed from patients dying from wasting diseases or sub-acute or chronic infections are falsely low due to "accidental" involution of the organ.

Absolute weights

AGE	V. SURY	HAMMAR	SCHRIDDE	RONCONI	KLOSE
years	*grams*	*grams*	*grams*	*grams*	*grams*
Newborn	14	13	13	10	9
1 to 5	22	23	17	16	15
6 to 10	29	26	20	21	18
11 to 15	32	38	25	28	25
16 to 20		26	20	20	20
21 to 25		25	19	18	17
26 to 35		20	14	14	14
36 to 45		16	10	13	9
46 to 55		13	7	9	6
56 to 65		10	4	12	5
66 to 75		6	3	5	4

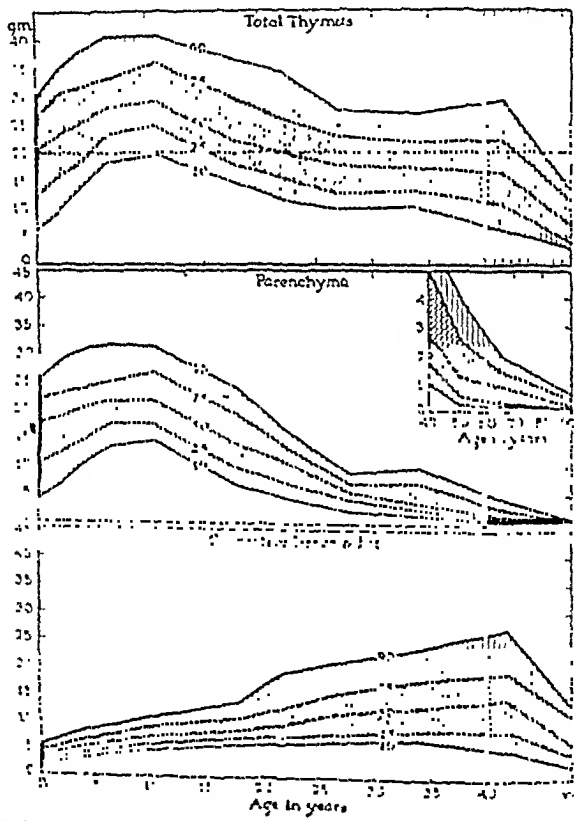


FIG. 3[After Boyd (12)]. Norms of the age trend and variability in weight of the whole thymus and of its parenchyma and connective tissue from birth to old age. The number on each line indicates the percentage expected below or on that line. The heavy broken line at 30 grams on the upper graph indicates the previously supposed upper limit of normal weight.

Boyd (12) has made an excellent biometric study, based upon Hammar's data, on 207 cases of accidental death within 24 hours of injury. Figures 3, 4 and 5, which are reproduced from her paper, embody her results on the development of the normal thymus and its component parts.

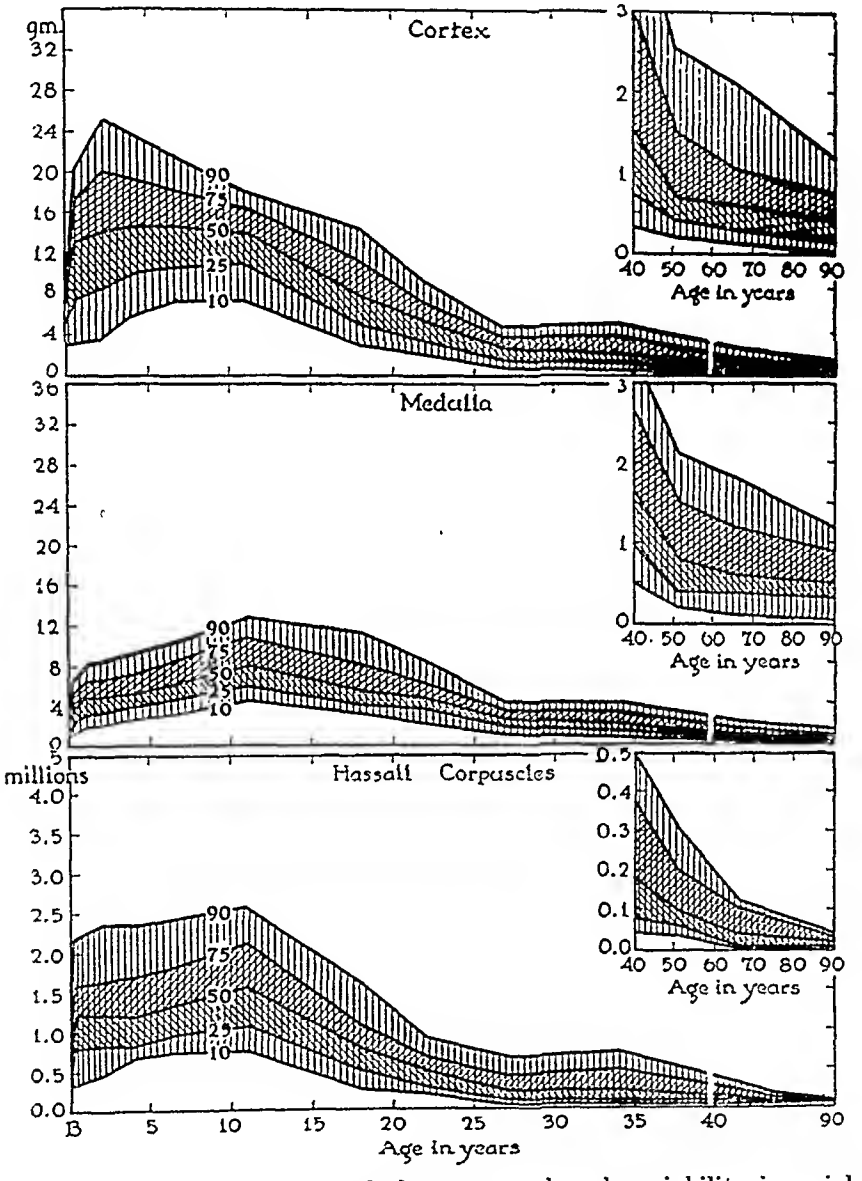


FIG. 4 [After Boyd (12)]. Norms of the age trend and variability in weight of the cortex and medulla and in the number of Hassall corpuscles from birth to old age. The number on each line indicates the percentage expected below or on that line.

It will be seen that the thymus is largest at about the time of puberty, following which it diminishes in size. It should be noted, however, that relative to total body weight, the organ is largest during the last few months of fetal life and at birth. Castaldi (15) computed that the coefficient of correlation of the

thymic weight in function of the bodily weight was highest during the fetal period (R = 0.837). This might possibly have some functional significance.

The medulla with its Hassall corpuscles begins, like the whole thymus, to involute at about the age of puberty. Involution of the lymphoid tissue in the cortex begins at 4 years of age.

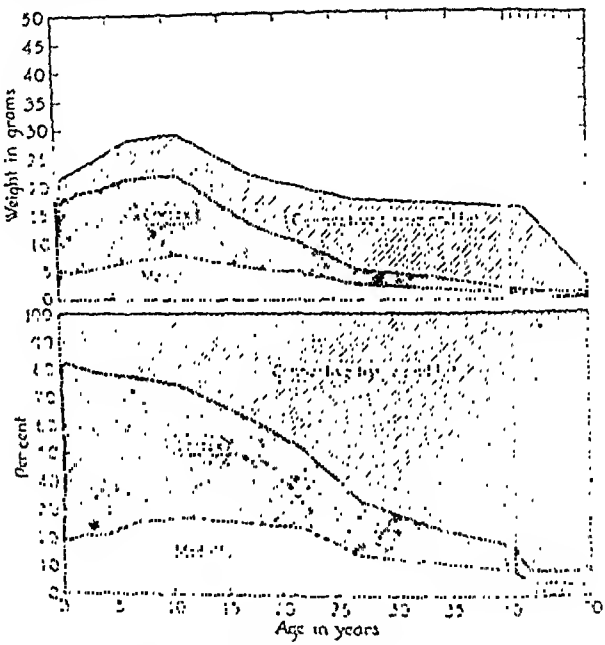


FIG. 5 [After Boyd (12)]. Absolute and relative medium weights of the component parts of the thymus from birth to old age.

C. "Accidental" Involution and Cellular Structure of Thymus

In addition to natural involution with increasing age and changes in size with altered function of certain endocrine glands previously described, the thymus undergoes "accidental" involution from a variety of apparently non-specific, injurious causes. These include malnutrition, prolonged muscular effort, exposure to cold, infections and poisoning by various harmful substances. Even these varied causes may have a common endocrine denominator since Selye (73) has shown that involution of the thymus does not occur under such circumstances in the absence of adrenal cortex tissue. This type of involution affects first the cortex and later the Hassall corpuscles, whilst the medulla is relatively spared.

Boyd (12) has made an excellent biometric study, based upon Hammar's data, on 207 cases of accidental death within 24 hours of injury. Figures 3, 4 and 5, which are reproduced from her paper, embody her results on the development of the normal thymus and its component parts.

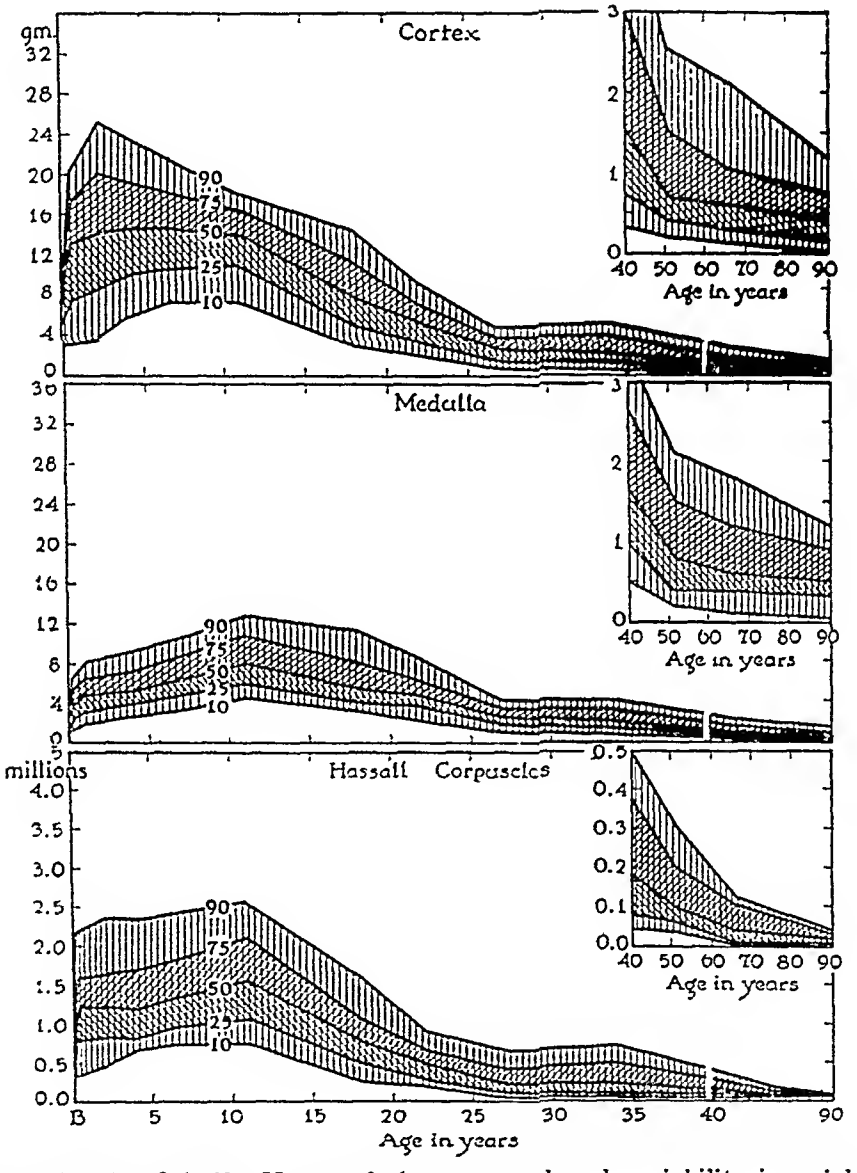
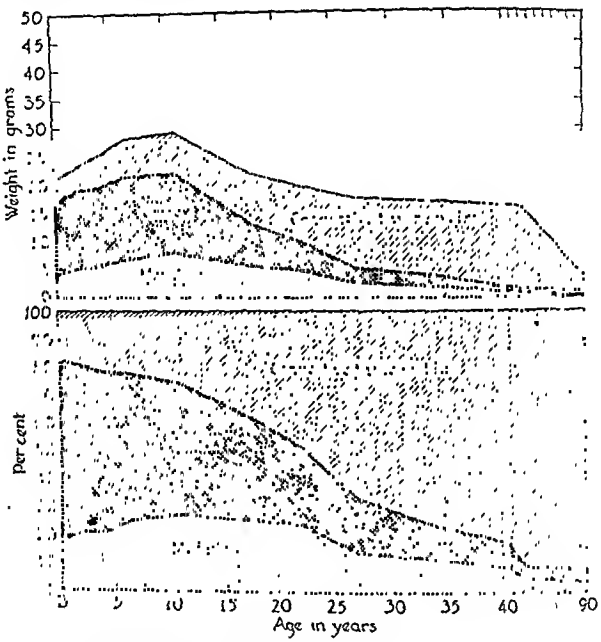


FIG. 4 [After Boyd (12)]. Norms of the age trend and variability in weight of the cortex and medulla and in the number of Hassall corpuscles from birth to old age. The number on each line indicates the percentage expected below or on that line.

It will be seen that the thymus is largest at about the time of puberty, following which it diminishes in size. It should be noted, however, that relative to total body weight, the organ is largest during the last few months of fetal life and at birth. Castaldi (15) computed that the coefficient of correlation of the

thymic weight in function of the bodily weight was highest during the fetal period (R = 0.837). This might possibly have some functional significance.

The medulla with its Hassall corpuscles begins, like the whole thymus, to involute at about the age of puberty. Involution of the lymphoid tissue in the cortex begins at 4 years of age.



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The cellular structure of the thymus, so carefully studied by Hammar (30), offers equal comfort both to those who claim a secretory function for the organ and to those who deny it. The medulla consists chiefly of large cells of epithelial origin which might conceivably elaborate an internal secretion. The cortex is made up largely of lymphocytes which seem to have their origin in common with similar cells elsewhere in the body. Hassall corpuscles, which increase in number with age, are of ectodermal origin according to Norris (62).

6. THEORETICAL CONSIDERATIONS

If myasthenia gravis is assumed to be due to thymic hyperfunction there should be some hypothesis to explain the normal function of the organ. This might be that the thymus, through the elaboration of some curare-like substance, acts as a brake to excessive or undesirable muscular activity. Even with a stretch of the imagination there is little evidence for such a theory but there is room for speculation. If the thymus were most active during fetal life there would be some reason for thinking that it might serve to reduce muscular activity in utero. Activity of the fetus begins at about the 5th month, increases to the 7th, and decreases thereafter until the time of birth. During the latter period of relative quiescence the anatomical substratum for muscular activity is present, as evidenced by normal crying, respiration, swallowing and somatic activity of babies born prematurely. Some form of chemical brake to muscular activity provides just as good an explanation for the relative quiescence of the fetus as the current idea that the unborn baby remains relatively quiet simply because it is warm and comfortable. In fact, the results of Twitty's experiments (see below) could lead us to believe that both explanations might be true, and that the action of the brake depends upon the temperature. Numerous direct observations by Barcroft and his co-workers (6), Windle (88) and others on animal fetuses show that the mechanism for neuromuscular activity is present long before birth but is very little evident under normal circumstances.

Windle (87) noted that reflex (neuromuscular) responses in cat embryos exhibited fatigue to a degree not seen with direct stimulation of muscle. Gonzales (24) curarized rat fetuses and found that reflex responses were abolished whereas direct muscle excitability was retained. He also showed (23) that direct muscle responses have a long latent period in very young but uncurarized rat fetuses. Krasnogorski (49) observed a long latent period (31.6 sigma) in striated muscle of the premature baby.

No curare-like substance is known to exist in the tissues of humans or of warm-blooded animals. Twitty and Johnson (80) have described, however, an interesting example of neuro-muscular block in salamanders. If embryonic tissue from *Triturus torsus* is removed at an early stage of growth (before absorption of the yolk sac) and grafted to an adult *Amblystoma punctatum* the recipient animal becomes paralyzed. By exquisite surgical experiments Twitty (79) has shown that the paralysis is due to some form of neural block and not to paralysis of the muscles directly. Aqueous extracts of Triturus embryos or of ovaran eggs caused similar paralysis when injected into Amblystoma. The paralyzing

agent was also found in adult Triturus female blood and the suggestion was made that its presence there might be correlated with periods during which it was being deposited in the developing ova. In view of the greatly increased activity of warm-blooded fetuses immediately after birth, it is of interest that Twitty's animals, which were paralyzed at room temperature, recovered their muscular movements rapidly when exposed to a cooler atmosphere and became paralyzed again on return to warmth.

7. POSSIBLE REASONS FOR FAILURE TO DEMONSTRATE A THYMUS HORMONE

If an endocrine function is proposed for the thymus some possible explanation should be offered for the failure of classical methods of endocrinology to demonstrate this function. This can be considered under two headings: extirpation experiments, and those dealing with administration of thymus substance or extracts.

A. Extirpation Experiments

Although some experiments have been vitiated by incomplete removal of the thymus with resulting regrowth of the organ, numerous carefully controlled extirpations have been made without any demonstrable effect upon the animals. The following theoretical explanations for failure might be offered:

1. The thymus might elaborate two antagonistic hormones which in health preserve a balance, and whose effects cancel each other in a removal experiment. A partial analogy can be offered in the case of early experiments on the pancreas where the islet hormone was destroyed, during extraction, by the digestive juices of the gland. Perhaps a better parallel can be found in the reciprocal effects of Sympathin and acetyl choline, although the activity of substances so widespread in the tissues is not susceptible of dissection by "removal" experiments. The failure of diabetes to develop in pancreatectomized animals which have also been deprived of the hypophysis or adrenal cortex is another example in point.
2. The thymus might be a secretory organ only during fetal life. So far as we are aware, extirpation has been done only on animals after birth.
3. The thymus might have a homeostatic function, the effect of its removal becoming apparent only if the animal were placed under special stress. An example of this is found in the case of the adrenal medulla, removal of which causes no detectable symptoms unless the animal is placed under conditions of stress.

B. Administration Experiments

Thymus has been administered to animals by feeding of the tissue, injection of various types of extracts and by surgical implantation. No definite proof of secretory activity of the organ has resulted from these experiments. Failure might be ascribed to the following causes:

1. Two antagonistic hormones might be elaborated by the thymus and cancel out each other on administration to animals.

2. The thymus might be a secretory organ only during fetal life. Administration of fetal thymus, or extracts of it, has apparently not been attempted.

3. It could be due to rapid destruction of hormone during removal of the thymus, incorrect methods of extraction for the winning of the active principle or non-absorption from the gastro-intestinal tract.

8. CLINICAL EXPERIENCE WITH MYASTHENIA GRAVIS

A. X-ray Evidence of Abnormality of Thymus

Clinical evidence of hypertrophy or tumor formation of the thymus is rare, even though necropsy experience leads to anticipation of abnormality of the organ in about half the cases. In 18 consecutive cases studied by us no abnormality was detected although roentgenograms of the chest were made in all, and special stereoscopic lateral and oblique views in some. Four of these patients died and came to autopsy. Thymus tumor was found in one, thymus hyperplasia in another, while two showed no thymic abnormality. Failure can often be laid to difficulty in outlining a tumor against the dense structures of the heart and mediastinum. Viets (81) studied 85 consecutive patients and found only in the last one of these a mediastinal tumor.

B. Effect of X-ray Therapy Directed to the Thymus

Only sporadic reports are available to judge the effect of this form of therapy. No conclusions can be drawn from them. In some instances there has been no amelioration of the disease, in others apparent remission has resulted (Eaton (21)). The natural tendency to remissions in this disease is a confusing factor. Nor can lack of improvement following radiation therapy be construed as evidence against hyperfunction of the thymus, since encapsulated adenomas would not be expected to prove sensitive to such treatment. Certainly adenomas of the thyroid, parathyroid or adrenal medulla are little if at all affected by X-ray therapy. Kennedy (43) reports some good results but no cures. Viets (82) found no improvement in over 30 cases treated by X-ray. Two of our cases were given X-ray therapy. One patient became worse during the second week of treatment and then slowly improved, which may be significant. The second patient showed no improvement. Hyland (39) reported improvement in two of four patients treated with X-ray. Grinker (26) observed improvement in one of two cases treated by roentgenotherapy.

C. Relationship of Myasthenia Gravis to Known Endocrine Disturbances

Myasthenia gravis does not appear to be connected with endocrine dysfunction except during pregnancy or in relation to the menses or to thyroid activity. The development of pregnancy in myasthenic women often leads to remission of the disease. Viets, Schwab and Brazier (83, 84) have made the best report on this aspect of the subject. Relapse occasionally occurs in the first three months but remission, often complete, is the rule in the last two trimesters. The remission may last for months thereafter. Sometimes remission occurs very early, even before the first menstrual period is missed. Examples to the contrary have

been reported (44, 51). Meyer (54) reported two cases in which myasthenia gravis developed and became intensified respectively after artificial menopause.

Three of our women patients regularly became worse at the time of their menstrual periods. One of these enjoyed a period of particularly good health during three months of unexplained amenorrhoea. Viets (82) described a patient who improved a week or two before missing a menstrual period.

A curious relationship seems to exist between hyperthyroidism and myasthenia gravis. The two conditions occasionally co-exist but perhaps not with greater than chance frequency. In 1932 Cohen and King (18) reported a case and reviewed the literature. They did not report on variations of the myasthenic state in relation to severity of hyperthyroidism.

Treatment of the hyperthyroidism may result in intensification of myasthenic symptoms. In one of our two cases with the two coincident conditions, hyperthyroidism was marked, whereas myasthenia was mild and only evidenced by some ptosis of the eyelids and weakness of jaw muscles during extreme bodily fatigue. Seven days after thyroidectomy, when symptoms of hyperthyroidism had disappeared and the B. M. R. was normal, full-blown myasthenia gravis appeared with ptosis, diplopia, facial weakness and difficulty in phonation, mastication and swallowing. Response to prostigmine was excellent.

Nevin (59) found that large doses of thyroid aggravate myasthenia gravis. Eaton (21)confirmed this in one case. A few cases of uncomplicated myasthenia gravis are said to have improved with thyroid administration although unpleasant symptoms of hyperthyroidism limited the therapeutic use of the procedure.

In the case described by Thorn and Tierney (76) myasthenic symptoms seem to have improved but little following subtotal thyroidectomy. Kowallis et al. (48) observed disappearance of hyperthyroidism and myasthenia gravis in a case submitted to subtotal thyroidectomy. It is noteworthy that after 17 days of preoperative iodine therapy the muscular weakness seemed to be intensified. Of five trials of iodine therapy in 3 additional cases improvement occurred on three occasions. Kowallis et al. refer to 12 other cases of combined hyperthyroidism and myasthenia gravis which have appeared in the literature.

Thorner (77) recently reviewed the evidence for this type of "see-saw" balance between myasthenia gravis and exophthalmic goiter. He described a case of myasthenia gravis in which the development of hyperthyroidism was attended by lessening of myasthenic symptoms. He referred to 2 previously reported cases in which myasthenia gravis developed after treatment for hyperthyroidism.

Moehlig (56) reported improvement following implantation of pellets of desoxycorticosterone acetate in one case. The report is not convincing. Intramuscular injections of D.C.A. aggravated myasthenic symptoms in one of our cases. Thorn and Tierney (76) observed no improvement in a case of combined hyperthyroidism and myasthenia gravis after prolonged administration of adrenal cortical extract.

The muscular weakness of Addison's disease has been [illegible] but is probably quite different in mechanism. There is

any predominant affection of muscles innervated from the brain stem and without actual paralysis from fatigue. Little has been published on the therapeutic use of sex hormones in myasthenia gravis. Thymic extract (Rowntree) was found to be ineffective by Viets (83).

D. Surgical Removal of Thymus

Attempts to influence the course of myasthenia gravis by extirpation of the thymus have been few. Data on the published cases are assembled in the following table:

AUTHOR	AGE	SEX	OPERATION	SIZE THYMUS OR TUMOR	RESULT
Schumacher & Roth 1912 (71) (Sauerbruch)	21	F	Thymectomy neck	Thymus enlarged 49 gms.	Improved
Haberer 1917 (29)	27	M	Partial thymectomy neck	Thymus involuted	Improved
Adler 1937 (1) (Sauerbruch)			Removal tumor thoracotomy	Benign tumor "child's head"	Died 8th day mediastinitis
Obiditsch 1937 (64) (Sauerbruch)				Tumor "man's fist"	Died 5th day streptococcus infection
Blalock et al. 1939 (8)			Removal tumor thoracotomy		Well after 4 years
Campbell et al. 1941 (14)	45	F	Removal tumor thoracotomy		Improved
Campbell et al. 1941 (14)	54	F	Removal tumor thoracotomy	Tumor small grapefruit 122 gms.	Died after 2 days, resp. failure
Blalock et al. 1941 (9)	33	M	Thymectomy thoracotomy	Thymus enlarged	Improved
Blalock et al. 1941 (9)	28	F	Thymectomy thoracotomy	Thymus enlarged	Improved
Blalock et al. 1941 (9)	22	F	Thymectomy thoracotomy	Thymus enlarged	Improved
Blalock et al. 1941 (9)	39	F	Thymectomy thoracotomy	Thymus enlarged	Died after 36 hours
Blalock et al. 1941 (9)	30	F	Thymectomy thoracotomy	Thymus enlarged	Improved
Blalock et al. 1941 (9)	34	F	Thymectomy thoracotomy	Thymus enlarged	?Improved
Eaton 1942 (21) (Clagett)				Tumor 70 gms.	Improved

The first seven cases recorded in the table were operated upon because of a presumed or demonstrated mediastinal tumor. The last six cases reported by Blalock et al. (9) were subjected to operation despite lack of clinical or roentgenographic evidence of mediastinal tumor.

All of the cases which have survived operation have shown improvement, in some instances amounting to cure. This is a striking fact. Nevertheless, complete disappearance of myasthenic symptoms has not resulted in all despite apparently complete thymectomy. This is disturbing but does not negate the positive results obtained.

E. Attempts to Demonstrate a Curare-like Substance in Myasthenia Gravis

A curare-like substance might be sought for in the blood or urine of patients with myasthenia gravis, or in extracts of their thymi removed at operation or at autopsy. No such attempt has been recorded. Experiments of this nature have been carried out in a few cases with Dr. Guy Odom and Dr. Wolfgang Klemperer. The results have so far been negative but deserve recording.

The main theoretical difficulties are as follows:

A. Time factor. It is impossible to know whether the hypothetical curare-like substance would act quickly or slowly, in terms of minutes or weeks. The only suggestive evidence is derived from patients with myasthenia gravis who have been subjected to thymectomy. It would seem that improvement occurs slowly in terms of weeks, and not immediately as in patients with tumors of the adrenal medulla or of the pancreatic islets. This would suggest a slowly acting substance such, for example, as the thyroid hormone, which acts over periods of days and weeks.

B. Derivation of substance. The hypothetical substance might not be present in blood or urine, or it might exist in infinitesimal amount. The substance might break down quickly during extraction from the thymus, or it might not be extracted by the solvents used.

C. Methods of assay. Little is known of how to test for such a substance. We envisaged the following four possibilities as evidence of curariform or "myasthenic" activity after administration to animals:

1. General evidence of muscular weakness or fatigability in animals, with reversal by prostigmine.
2. Neuro-muscular block in frogs (curare effect of Claude Bernard).
3. Neuro-myographic defects (Briscoe (13)) in cats.
4. Increase of creatinuria in rats.

9. EXPERIMENTS

Urine extracts. Several litres of urine were collected over several days from each of 9 patients with myasthenia gravis. Each specimen was concentrated in vacuo at 45°C and extracted with ether (continuous) or olive oil (continuous stirring) overnight. Ether extract dispersed in water, 1 cc. = 250 to 500 cc. original urine.

These extracts were tested by injection into frogs according to the classic method of Claude Bernard. The motor nerve or the muscle was stimulated by a Harvard inductorium. The ether extract from one patient seemed to have a curare-like effect. Experiments with all other extracts were negative.

Six tests of three ether extracts of urine from patients with myasthenia gravis were made on the cats' nerve-muscle preparation described by Briscoe (13). Injections were given intravenously. No curariform effect on the myogram was obtained. Control experiments with curarine resulted in typical myographic changes.

Blood. The cats' nerve-muscle preparation was also used for these tests. Myograms were recorded during high and low rates of stimulation of the motor

nerve. Blood was obtained from a patient with severe myasthenia gravis (no recent prostigmine) and was injected into the animal with a delay of only several minutes. At various times the following observations were made:

10 cc. defibrinated venous blood injected intravenously—no curariform effect.

25 cc. defibrinated venous blood injected intraperitoneally 18 hours prior to test—no curariform effect.

5 cc. fresh venous blood injected intravenously—no curariform effect.

5 cc. laked (freezing) oxalated blood injected intravenously—no curariform effect.

7 cc. defibrinated arterial blood injected intravenously—faint curariform effect.

11 cc. oxalated arterial blood injected intravenously—no curariform effect.

15 cc. laked arterial blood injected intravenously—no curariform effect.

8 cc. fresh arterial blood injected intravenously—no curariform effect.

Extracts of thymic tumors. We have had an opportunity of making extracts of two tumors immediately after their removal by Dr. Eldridge Campbell (14) from patients with myasthenia gravis. The tumors were macerated and extracted in N/10 HCl and later with alcohol and with ether. We have also extracted a tumor removed from one of our patients at necropsy.

The extracts were tested on the cats' nerve-muscle preparation previously mentioned. No curariform activity could be demonstrated.

Cyst fluid from one tumor and extracts of another were injected into rats daily for periods up to two weeks with no obvious change in their behavior.

Extracts of calf thymus. Numerous experiments have been carried out with Miss Doris Brophy on aqueous, alcoholic and ether extracts of thymuses from calves. Some of these extracts have been highly concentrated (1 cc. = 50 gms. fresh tissue). The extracts were injected daily into rats and daily excretion of creatine and creatinine were followed over several weeks. They caused great increase of creatine excretion but control extracts prepared from calves' brain tissue showed similar properties. No symptoms suggesting curarization were observed. The above experiments will be reported in full elsewhere.

10. SUMMARY

The general character of myasthenia gravis suggests an endocrine disorder. No important structural changes occur in the nerves or muscles and there is a tendency to remissions and exacerbations, sometimes due to changes in function of known endocrine glands.

Tumor or hyperplasia of the thymus is found at necropsy in over half the cases. It is possible that microscopic search for aberrant or very small nodules might increase the percentage. The tumors are almost invariably benign and encapsulated adenomas. Other conditions associated with thymic hyperplasia are discussed. It is pointed out that hyperthyroidism, adrenal cortex deficiency and castration each have in common the triad: muscular asthenia, creatinuria and hypertrophy of the thymus.

The symptoms of myasthenia gravis are almost identical with those of prolonged curare poisoning and the underlying neuro-muscular disturbance is in both cases a peripheral phenomenon. The beneficial effects of prostigmine and the aggravation of symptoms by curariform drugs have led to the suspicion that there is some fault of chemical transmission at the myo-neural junction. The weight of evidence would favor the action of some curare-like substance.

There is still no convincing experimental evidence of an endocrine function for the thymus, although a number of findings are suggestive. Under both clinical and experimental conditions the thymus undergoes hypertrophy during states of hyperthyroidism or with hypofunction of the adrenal cortex or sex glands. Reverse conditions cause involution of the thymus. The thymus is derived from the ectoderm of the cervical sinus and from the endoderm of the third branchial complex, which also gives rise to the parathyroids. Although the organ is largest at about the time of puberty, its weight in proportion to total body weight is greatest during the last months of fetal life and at birth. The thymus might conceivably produce some substance which acts as a chemical brake to muscular activity, especially during fetal development.

X-ray evidence of thymic enlargement or tumor is rare in myasthenia gravis, although necropsy experience leads to anticipation of abnormality of the organ in about half the cases. X-ray therapy directed to the thymus has yielded inconclusive results, although some patients seem to have benefited. Remissions and exacerbations of the disease, in relation to pregnancy and to alteration of the thyroid function, are of great interest. A tabulation is made of 13 cases of myasthenia gravis in which operation has been performed for removal of the thymus or of a thymic tumor. All of the patients who survived operation appeared to show some improvement, in a few instances amounting to cure. Nevertheless, complete disappearance of myasthenic symptoms did not result in all cases despite apparently complete thymectomy.

Some experiments are described which were aimed at the demonstration of a curare-like substance in the blood and urine of myasthenic patients and in extracts of thymic tumors removed at operation or autopsy. No such substance could be demonstrated. It is concluded that further search should be made for such a substance and that further thymectomies should be carried out in certain carefully selected cases.

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THE PRESENT STATUS OF NON-OBSTRUCTIVE JAUNDICE DUE TO INFECTIOUS AND CHEMICAL AGENTS

Causative Agents, Pathogenesis, Inter-relationships, Clinical Characteristics

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Disease of the hepatic parenchyma is due either to infections,—directly or indirectly,—or to chemical agents. The clinical features help little in determining the etiology of a given case. Neither do the new tests of hepatic function. For example, the manifestations of (1) simple (or catarrhal) jaundice, of (2) jaundice following the use of cinchophen or arsphenamine and of (3) the jaundice which occasionally appears in early secondary syphilis are similar. Most forms of acute hepatic degeneration may progress to a fatal issue with the clinical and histologic characteristics of acute yellow atrophy.

Part I

INFECTIOUS FORMS OF JAUNDICE

The following is a tentative classification of those hepatic degenerations attributed to infections:

Simple Jaundice ("Catarrhal" Jaundice)

Simple or "catarrhal" jaundice is taken as the paradigm of the forms of hepatic degeneration. However, in spite of its relative clinical uniformity, there is doubt whether it is a nosologic entity or includes several diseases of different causes. It therefore deserves careful analysis. The term "simple jaundice" is preferable. Synonyms, such as "epidemic jaundice" and "infectious jaundice" are equivocal, being just as applicable to spirochetal jaundice or to yellow fever. The term "toxic hepatitis" is often applied indiscriminately and should be discarded.

Epidemiology: Simple jaundice has been known since antiquity in both sporadic and epidemic form. It was frequently confused with Weil's disease (1880) until 1898, when Quincke and Hoppe-Seyler differentiated these two diseases. Cockayne (1) in 1912, presented the thesis that simple jaundice is an infectious disease and essentially uniform, whether epidemic or sporadic.

Pickles' (2), careful analysis of the two Yorkshire epidemics of 1929 and 1935 helped clarify the epidemiology of the disease. The first epidemic distinctly began with an outbreak at school and finally involved 250 cases in one valley with a population of 5700. The four-week interval between cases in families suggested that the infective period of any one case is short, otherwise the periodicity would not have been preserved. Close personal contact appeared necessary for transmission of the disease.

We recently saw several cases from an epidemic in an orphan asylum (Chart II). The dates suggest that several groups of cases which occurred on closely succeeding days were really infected from a common source, while the true in-

cubation periods were represented by some of the longer intervals, 17, 21, 25, and 28 days, during which no fresh cases occurred.

CHART I

Infectious Causes of Hepatic Parenchymal Disease

A. Infections in which hepatic degeneration is a dominant feature
   1) a. Simple (or Catarrhal) Jaundice (sporadic or epidemic: Etiology unknown)
      b. Acute Yellow Atrophy (sporadic or epidemic: Etiology unknown)
   2) Leptospirosis: Spirochetal jaundice (due to various leptospirae)
   3) Yellow Fever (filtrable virus)

B. General infections in which jaundice occasionally is an incident
   1) Lobar pneumonia and pneumococcus peritonitis
   2) Infections with Streptococcus, Welch bacillus, Spirocheta recurrentis, dysentery bacilli, typhoid bacillus
   3) Syphilis
      a. congenital
      b. secondary
   4) Tuberculosis
   5) Infectious Mononucleosis
   6) Malaria
   7) Oroya Fever

CHART II

An Epidemic of Simple Jaundice in an Orphan Asylum

(Courtesy of Dr. Averbuck)

DATE OF ONSET OF JAUNDICE (1934)	NAME	INTERVAL
		days
5/3	S. D.	10
5/13	H. K.	
5/15	J. N.	
5/17	J. A.	
5/17	M. H.	8
5/25	R. M.	
6/15	F. E.	21
6/26	H. F.	11
6/27	N. G.	17
7/14	A. L.	
7/16	M. L.	
7/17	M. K.	
7/22	M. D.	
7/23	A. S.	10
8/3	S. C.	
8/31	G. L.	28
8/31	S. A.	
9/1	S. A.	
9/1	O. M.	25
9/26	M. V.	

n the epidemic reported by Hiscock and Rogers (3) at Yale University, 65 ›s occurred explosively within fifteen days, in students in close contact with ı other, 72 per cent in the freshman class. Beauchamps (4) reported three ›s in a family at approximately four week periods.

Ve recently encountered an instance in which the patient became ill four ks after exposure to a cousin who several days later developed sub-acute ow atrophy. The incubation period in this case therefore was four weeks.

Besides the high incidence of cases reported by Pickles, de Bettancourt and Silva (5) describe an epidemic of typical simple jaundice which affected 200 of 860 inhabitants of a small village in Portugal and spread to involve neighing villages situated along the same waterway. As Pickles pointed out, the al relationships in these village groups are very close and may account for incidence, as transmission by water, food, and animal or insect vectors has yet not been proved.

Generally, however, only a small number of exposed persons acquires the inion as in the epidemics described by Blumer (6) at Yale, Bergstrand (21) in ›den, Morgan and Brown (7) in England. Apparently, simple jaundice ›es an immunity of long duration. Thus in the Surrey epidemic of 1930, dlay, Dunlop and Brown (8) noted that jaundice skipped those schools which suffered from severe outbreaks of jaundice in the preceding epidemic of 1927. Cockayne's suggestion to explain the long interval between cases, that the inious agent remains alive in the room occupied by the patient, has not been stantiated. No case of transmission has been noted in a hospital ward. The dies of all the epidemics have shown that the patient's infectivity has ended the time jaundice appears.

Clinical Characteristics: The mode of onset varies in the different epidemics; re have been some in which almost every patient had upper respiratory sympıs (Blumer (6), Bamberger).

n our series of 39 cases of simple jaundice (sporadic type) 9 had acute upper piratory infections. One patient had diffuse abdominal pain preliminary to "grippe" and sinusitis for which he was hospitalized. Eleven days after onset of his severe respiratory infection, typical "catarrhal jaundice" apıred. This relationship of the upper respiratory infections to simple jaundice gests the occurrence of contact infection in the prodromal period by means nasal discharges.

There are scattered reports, beginning with Graves (9) in 1864, of cases in ich acute polyarthritis occurs in the prodromal period. Generally all the ›dromal symptoms except the gastrointestinal subside with the appearance jaundice.

Because of the prominence of the gastrointestinal prodromata, the old-time ıicians attributed "catarrhal" jaundice to dietary indiscretion. We have ınd pain confusing in the differential diagnosis between simple jaundice and ›lelithiasis. Of our 39 cases of simple jaundice, 25 had pain of varying de›e either in the right upper quadrant, the epigastrium or diffused over the lomen. Since most of these were also accompanied by nausea exacerbated

by fatty foods the diagnosis of cholelithiasis was occasionally considered. Many cases have no symptoms other than the jaundice itself.

Pickles (2) commented on the frequency of severe abdominal pain in the Yorkshire epidemic, so marked as occasionally to have suggested a perforated duodenal ulcer.

Itching, considered characteristic of obstructive jaundice and of value in differential diagnosis, was prominent in 12 of our 39 cases. Bradycardia, also long cited as characteristic of simple jaundice, was unusual in our cases. In one young man, the bradycardia appeared in the prodromal period when only high temperature and systemic symptoms were present and the diagnosis of typhoid fever was considered before the jaundice appeared.

There are many statements that the stools are never completely acholic; however, if they are examined frequently, transient acholia is occasionally present. In some mild cases the stools are normal throughout. During convalescence they regularly become darker, indicative of the increased urobilin excretion which follows the increased output of bile by the liver.

Frequently there is a leucopenia with lymphocytosis. Many observers have noticed leucocytosis in the first three or four days followed by leucopenia or relative monocytosis later. Lymphocytes are often 50 to 60 per cent, the monocytes over 10 per cent. Findlay and Dunlop (8) have noted the increase in lymphocytes and mononuclear cells. From their description the cells are similar to those found in many cases of infectious mononucleosis.

Eppinger (10) called attention to the frequent high red cell count ($5\frac{1}{2}$ or 6 million). We found hematocrit and hemoglobin to be high normal in 7 of 9 cases. The sedimentation time is normal or slightly prolonged.

Though clinical recovery is complete, several studies indicate that residual latent impairment of liver function does occur. Soffer and Paulson (11) using the bilirubin excretion test on 11 patients who had had simple jaundice several years previously, found that 9 of them had retention from 10 per cent to 50 per cent at the end of four hours. They think, therefore, that the latent hepatic damage is probably permanent.

Pathogenesis: Anatomic studies of the liver have been few. Obstruction of the opening of the ductus choledochus as part of "catarrah" of the gastrointestinal mucous membrane was suggested by Virchow (12) as the cause of "catarrhal jaundice", but in his paper he gave little specific clinical or anatomic evidence. In one case, frequently cited as evidence for the "mucus-plug" theory, the lesion actually was a mucocele of the common bile duct.

Klemperer, Killian and Heyd, in 1926, came to the conclusion that the only valid anatomic examinations in simple jaundice prior to their article were four necropsies made by Eppinger. Three of these were of soldiers who died of wound tetanus when they were already suffering from the jaundice which was then epidemic. The liver showed diffuse degeneration with multiple small foci of necrosis.

In Eppinger's fourth case there was, however, some evidence suggestive of an obstructive mechanism. The patient, a girl of nineteen, who committed sui-

cide during her attack of jaundice by leaping from the window, was found to have severe catarrhal changes of the mucosa of the stomach, duodenum, and common duct, and swelling of the lymphadenoid tissue about the common duct. Microscopically the bile capillaries were distended and occasionally ruptured. The gall bladder contained bile which could not be expressed. The intrahepatic ducts contained dark, tenacious bile. Parenchymal lesions were not described.

Klemperer (13) et al. reported a biopsy of liver from a woman with simple jaundice in whom the mistaken diagnosis of obstructive jaundice had been made. The normal liver architecture was retained except in scattered foci in which the liver cells were diffusely granular. In older foci only the reticular framework and proliferated endothelial cells were present. He supported Frerich's view (1858) that in many cases cell necrosis is present in simple jaundice which thus differs histologically from acute yellow atrophy only in degree.

Barber and Osborn (14) (1939) reported the morbid anatomy of a case of sporadic simple jaundice in which death had been accidental. The main lesion was in the hepatic cells around the central vein. The cells contained granules of bile pigment, mitoses, and hyperchromatic nuclei. Only the bile capillaries near the central veins were distended with bile. The gall bladder and common bile duct were collapsed and did not contain colored bile. The ampulla of Vater contained a loose plug of epithelial cells and debris, evidently not causing obstruction.

Klemperer suggested that the clinical term simple jaundice may apply to three different processes, first, and most frequently, parenchymatous hepatic degeneration; second, a cholangitis of hematogenous origin with obstruction of the fine biliary radicles; third, and hypothetically, a cholangitis due to an ascending infection from duodenitis with a plug of mucus in the papilla of Vater.

The problem of the pathogenesis of simple jaundice is at present unsolved.

Infectious Agents Causing Simple Jaundice: In spite of numerous bacteriologic studies, very little is known of a specific infectious agent as the cause of simple jaundice or of acute yellow atrophy. Blood cultures, duodenal cultures and animal inoculations have always been negative. There are scattered instances in which paratyphoid bacilli were cultured from stools or duodenal contents, or were agglutinated by the blood serum. However, these constitute a very small percentage of the cases in which investigations have been made (only about 3 per cent of the 1723 cases reported by Ruge (15)), and the fact that known paratyphoid infections are seldom characterized by jaundice, and that paratyphoid carriers are not rare, makes this a very improbable cause of simple jaundice.

From several far-flung sources has come evidence that different viruses may cause the apparently characteristic clinical picture of simple jaundice and acute yellow atrophy.

1) There is one case of possible laboratory infection which occurred in H. C. Brown (7) who handled a serum specimen from one of the Yorkshire cases in 1929 and developed typical simple jaundice, having had no other exposure.

2) In a British institution for mental defectives, seven children received pooled

convalescent measles serum and developed jaundice after 78 to 88 days. Four of them had typical simple jaundice. The other three died and autopsy showed acute yellow atrophy. Two months later, two other children who had been in contact with the first group of jaundiced children developed what appeared to be simple jaundice (16).

3) This led to investigation by the British Ministry of Health (17). They found that 109 persons had been inoculated with this pooled convalescent serum. Of these, 37 developed typical simple jaundice. Seven died. Bacteriologic and toxicologic investigation of the serum was completely negative. They point out that no similar results on a large scale had been previously reported although occasional jaundice two months or so after injection of human or foreign sera has occurred.

4) Another series of jaundice cases followed immunization against yellow fever (18). In this instance, an attenuated mouse brain virus, alone or mixed with immune human or animal serum, was used in 2200 persons. Forty-eight of these developed what appeared to be "common infective jaundice" two or three months after inoculation. That the disease was not yellow fever was strongly suggested by the clinical characteristics, by the fact that two persons had previously had yellow fever which always gives permanent immunity, and by the absence of the typical increase of yellow-fever immune bodies in their blood.

It thus appears that, since the mouse brain was the common factor in the variations of the modes of immunization, a contaminant virus may have been present.

5) The recent work of Andersen (19) of Copenhagen, if confirmed,* will be of greatest importance. He noted that epidemics of hepatitis, contrary to most epidemics, had a greater case-incidence in country villages than in crowded towns. He also learned that jaundice occurs as an epidemic in pigs, and that the regulations concerning the consumption of pork are more lax on the farms. On the basis of this clue, he performed various experiments. He transferred the pig disease through four generations of young pigs by feeding them liver of infected pigs. For successful transmission it was essential that the pigs were undernourished. He also succeeded in producing jaundice in pigs by feeding them 50 cc. of duodenal bile from human simple jaundice cases. The disease appeared to be the same as that transmitted from the pig epidemic and had the same incubation period of three to four days (quite different from the longer incubation in clinical simple jaundice). Grossly and microscopically the livers of these animals showed hepatic degeneration. No specific micro-organism was isolated.

From the widely different yet constant incubation periods in the human simple jaundice epidemics (3 to 5 weeks), in the outbreak from convalescent serum (8 to 9 weeks), in the yellow fever immunizations (8 to 12 weeks), and in Andersen's

* Blumer has recently confirmed these observations in the United States, reporting febrile simple jaundice occurring in two friends, only five days after eating roast pork, with recovery in about three weeks. (Blumer, G., Jaundice in Pigs and Human Beings. J. Mt. Sinai Hosp., 1942, 8, 418.)

mines, sewers, trenches and in industries that attract rats. It is, therefore, an industrial hazard of miners, sewer workers, butchers, fish handlers. The dog-carried type has occurred in sporadic cases among veterinarians and laboratory workers.

The different epidemiologic types are illustrated in the following reports:

Recently 40 cases of jaundice occurred among school children in Detroit (38). There was a definite history of person-to-person contact and a coincident outbreak of jaundice in kennels. Liver scrapings of the dogs showed spirochetes. One three-year old child, with a history of exposure to a dog convalescent from jaundice, developed gastro-intestinal and upper respiratory prodromata followed by jaundice, and died in three weeks. The blood serum agglutinated leptospirae icterohemorrhagica in a dilution of 1:30,000. Spirochetes were isolated from the blood and liver scrapings. If the intensive study of this case had not led to the discovery of spirochetes, the other cases might have been registered as simple jaundice.

Very recently an outbreak of leptospirosis occurred in Pennsylvania (35). Seven young men swam together in a pool contaminated by rats from a barn. Within four to twelve days all had become ill. Two developed very severe typical Weil's disease and the spirochetes were identified. One patient died and an autopsy was done. The other cases were mild and would have been regarded as grippe.

An epidemic of jaundice suggesting spirochetal jaundice was reported in 1936 in a religious novitiate (39). This consisted of three groups completely isolated from each other and from outsiders except for the use of water from the holy font. The first case occurred in a senior in September 1934 and quickly spread to others in his group. It was a month before there was any case in the junior group. Bacteriologic and epidemiologic studies disclosed a leptospira in the font, which was not pathogenic experimentally.

In the absence of jaundice, leptospirosis may be mistaken clinically for influenza or undulant fever. Only 50 per cent of cases of Weil's disease and 2 per cent of those of "field fever" are icteric and these icteric cases carry an especially grave prognosis.

Clinical Characteristics: After an incubation period of four to twelve days, an abrupt onset occurs with fever, prostration, vomiting, muscle cramps, conjunctivitis and occasionally with meningeal signs. Herpes labialis occurs in about 40 per cent and is invariably hemorrhagic. This febrile phase is associated with the circulation of leptospirae in the peripheral blood.

The toxic, afebrile phase, occurs after the fifth day and is characterized by acute nephritis, hemorrhages, and in 50 per cent, by jaundice. No spirochetes are in the blood stream then, though they are found in the parenchymal organs in fatal cases. Antibodies appear.

Convalescence occurs in the third week, or a febrile relapse may occur without spirochetes in the blood stream and without the previous symptoms. The hemorrhages and the jaundice are not related.

Diagnosis is greatly aided by laboratory tests,—in the first eight days by

It is generally accepted that the tendency to anasarca is connected with t low blood proteins.

It appears probable from consideration of pathogenesis and etiology th though cases of simple jaundice may proceed to atrophy, neither term represer a single disease.

Leptospirosis (Spirochetal Jaundice)

Spirochetal jaundice is accepted as a common disease in Europe and Asia, b it was considered rare in the United States because of the previous lack of sp cialized laboratory facilities and of elinical awareness of this disease. The fi American case was described by Wadsworth (34) in 1922; and by 1938 only had been noted, whereas 248 had occurred in Great Britain (35). Simple jau dice and Weil's disease were frequently eonfused until differentiated by Quinc and Hoppe-Seyler in 1898. In the tropies, Weil's disease has been confused wi yellow fever.

Leptospirosis includes a number of diseases related on the basis of both tl clinical pictures and the causative organisms, though varying in severity, in ge graphic distribution, and in the vectors. The most classical type, but not ne essarily the most frequent, is Weil's disease.

Epidemiology: The principal leptospirae are:

(1) L. icterohemorrhagica—Weil's disease—carried by rodents, especially rat and by dogs—to man.
(2) L. canicola—carried by and causing disease in dogs—transmissible to ma occurring in Holland, the United States.
(3) L. grippo—typhosa—causes an infection in man variously known "summer influenza," "field fever," "mud fever," "swamp fever," occurrir in Europe and Asia and in the United States.
(4) L. hebdomadis—"seven-day fever" of Japan.

It has been shown that at least 60 per cent of rats harbor leptospirae icter hemorrhagicae which are excreted in the urine. Another member, leptospi canicola, produces a canine disease which is very similar to human Weil's diseas and which is readily transmissible to man. About 10 per cent of a series of do tested in New York by Lederle Laboratories harbored one or the other type leptospira. Dogs after recovery still excrete spirochetes in the urine (36).

Guinea pigs are relatively less susceptible to leptospira icterohemorrhagic than other animals, which makes a negative response to inoculation of dimir ished diagnostic importance.

Because of the excretion of the organisms in human urine in one stage of th disease, person-to-person transmission may occur. Latent leptospirosis has bee demonstrated by agglutination tests in rat-catchers.

Infection in human beings occurs by means of contact with contaminate urine. The organisms may invade the mucous membranes or the abraded ski (37) (44). The distribution of the human disease follows from the distributio of the organism in lower animals. The rat-carried type occurs in sporadic case or in characteristically localized epidemics arising from contaminated pool

mines, sewers, trenches and in industries that attract rats. It is, therefore, an industrial hazard of miners, sewer workers, butchers, fish handlers. The dog-carried type has occurred in sporadic cases among veterinarians and laboratory workers.

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Diagnosis is greatly aided by laboratory tests,—in the first eight days by

guinea pig inoculations with the positive findings of spirochetes; of generalized hemorrhages in the viscera and lungs; and more reliably, by a serologic agglutination and lysis test of the patient's serum which is regarded as positive with a titer above 1:300 (40).

Therapeutic serum, effective chiefly in the pre-icteric stage, when diagnosis is most difficult, has been developed, but is not generally available.

The lesions are capillary damage, enlargement of the liver with proliferation of hepatic cells, acute nephritis, microscopic hemorrhages in the muscles and fairly regularly, hemorrhages in the adrenals (41).

The mechanism of jaundice in Weil's disease is not known. Subsequent cirrhosis has not been reported.

The following case summary is typical of the clinical course of Weil's disease:

Recently at the Mount Sinai Hospital, a forty-year old, white, sewer-cleaner, was admitted with a five-day history of malaise, fever, generalized muscle aches and prostration. He had seen many rats in the course of his professional pursuits, but had never been bitten by any. He had had nausea and vomiting and a diarrhea of black, watery stools. Jaundice was noted for the first time five days after the onset.

At his initial examination, his blood pressure was 80 systolic and 45 diastolic, his heart rate was 140, his pulse rate 120, and the temperature 97.2°F. His liver was enlarged, the spleen was not palpable. A few discrete lymph nodes were present in the axillae. Some purpuric spots were present over the left knee. His urine contained much albumin, red blood cells and white blood cells. His coffee-ground vomitus and black-brown stools were guaiac-positive. He became anuric. His blood urea was 112 mg. per 100 cc. The blood cholesterol was 185 with the esters 45. Guinea pig inoculation was positive for the leptospira icterohemorrhagica.

He lapsed into stupor and died on the eighth day of his illness. Post-mortem examination showed the spirochetes in most of the organs. The esophagus had large patches of hemorrhagic ulceration. The kidneys showed parenchymatous degeneration. The liver was grossly normal. Microscopically there was diffuse destruction of the sinusoidal architecture, the hepatic cells were arranged in disorderly fashion and showed frequent mitoses. A few were necrotic. There were focal hemorrhages and periportal accumulations of lymphocytes and polymorphonuclear cells. The Kupffer cells showed erythrophagocytosis.

Yellow Fever

The astonishing recent advance of our knowledge and control of yellow fever has been summarized by Sawyer (42). A number of important developments hinge on the discovery that the disease is due to a virus easily transmitted to certain species of monkeys and to white mice, and readily grown on chick embryo tissue cultures:

(1) Recovered cases can be identified by the power of their serum to protect inoculated mice. Aged men who had the disease in infancy still show this protective power.

(2) By this means many mild or asymptomatic cases which would otherwise not have been diagnosed are now detected in endemic areas.

(3) By tests of populations *en masse* the Rockefeller Foundation workers have charted two vast endemic reservoirs of the disease, the one in the tropical forests

of the Amazon basin, the other in western tropical Africa. It is certain that there the disease is kept alive in monkeys and perhaps in other animals.

(4) Protective immunization has been the most important advance. This depends on loss of human virulence by a strain of virus after prolonged growth in tissue cultures. At first this virus was combined with protective serum but it is now safely administered without the serum. Protective antibodies develop within three weeks in over 90 per cent of vaccinated persons. In Brazil over a million people have been vaccinated since 1938 and the United States War Department now vaccinates all military personnel in the tropics. Vaccine is not yet commercially available.

In recent years careful quarantine has prevented outbreaks such as were formerly common in the various great ports of the temperate zone (43). In 1940 a ship from Dakar, Africa, reached England with a number of cases which had been mistaken for food poisoning.

Recent experiments have shown that the virus can be transmitted by several species of mosquitoes other than aedes aegypti (stegomeia). Great outbreaks would easily occur again since there are many parts of the world, at present uninfected, which harbor mosquitoes capable of transmitting the disease. In considering the possibility of conveyance of the disease by aeroplanes and other rapid means of transport it is important to remember the fact, known already to Reed and his associates, that there is a period of twelve days before infected mosquitoes can transmit the disease.

The disease has been produced by placing infectious monkey blood on the intact skin of monkeys. This partially explains the great danger to laboratory workers, of whom over thirty have developed the disease.

The disease is sharply divisible into two periods: The first or "stage of active congestion" lasts three or four days and is characterized by extreme prostration, fever, headaches, backache, but no jaundice. It is only during this first "no-jaundice" stage that the blood of the patient is infectious. The second stage, classically the "stage of stasis", begins about the third or fourth day as the temperature drops to normal. At this time jaundice appears and in typical cases progresses to an extreme degree. There are hemorrhagic manifestations and progressive albuminuria; suppression of urine may occur. This stage of kidney and liver symptoms lasts one or two weeks.

The symptoms have been confused with those of Weil's disease. This is illustrated by the unfortunate experience of Noguchi, who, attracted to South America by an alleged epidemic of yellow fever, isolated a leptospira, which is now thought to have been the leptospira icterohemorrhagica of Weil's disease.

In fatal cases the liver shows profound necrosis and fatty degeneration. The peculiar accentuation of this in the midzonal portion of the lobule is said by Rocha-Lima to be characteristic. The kidneys show necrosis and fatty degeneration in the tubular epithelial cells. It is remarkable, in view of the presence of extensive lesions which must be assumed to account for the clinical severity, that cases which recover are said to show no sequellae either from liver or kidneys.

SYSTEMIC INFECTIONS ASSOCIATED WITH JAUNDICE

In this group, jaundice is only an occasional incident and not the essential disease. It includes pneumococcus infections, general septicemias, secondary syphilis, malaria, oroya fever, acute infectious mononucleosis, tuberculosis, typhoid fever and rarely, relapsing fever, and Rocky Mountain spotted fever. The mechanisms of jaundice in this miscellaneous group are probably varied. The function of the liver as the chief organ for removal of bacterial and toxic substances from the blood may be a factor contributing to its vulnerability (45).

Lobar Pneumonia and Pneumococcus Peritonitis

Subicterus is characteristie of lobar pneumonia and is due to several mechanisms which occur either alone or in combination. It is associated with demonstrably impaired liver function which is transitory in the recovered cases, and, if persistent, is of grave prognosis (46). The causes proposed are:

1) Liver cell damage from anoxemia (Rich (47))
2) Hemolysis in the consolidated lung
3) Direct effect of pneumococcic toxins on red blood cells in circulation
4) Disintegration of red blood cells due to hemostasis (Ham and Castle (48)).

Severe jaundice is a rare complication of grave import occurring most frequently in cases with positive blood cultures, Birch (49) reports two cases among 860 of both broncho- and lobar pneumonia. It is probable that the bacteremia rather than the local pulmonary lesion, accounts for the jaundice. This is suggested by the occurrence of jaundice in two cases of pneumococcus peritonitis with bacteremia in which the post-mortem examination showed diffuse liver degeneration (50). Baehr and Klemperer have stressed the point that jaundice occurs more frequently in pneumococcic bacteremia than in any other type of bacteremia, even in the absence of pneumonia, and conclude that the mechanism is the bacteremia with the characteristic of bile-solubility of the organisms. They minimize the importance of the hemolytic action of the organism, since streptococcus hemolyticus bacteremia is less frequently associated with jaundice of similar intensity.

General Septicemias

In acute septicemias, jaundice of varying degree is an occasional symptom and generally of unfavorable prognosis. The organisms commonly concerned are the pneumococcus, hemolytic streptococcus, staphylococcus, and the Welch bacillus. Many of the cases of severe jaundice in the new-born belong in this group.

Bingold (51) found 28 cases of frank jaundice among 200 of septicemia. Jaundice is rare in staphylococcus aureus septicemia, more common in hemolytic streptococcus infections. It is most common in anaerobic septicemias, the most important being anaerobie streptococcus and the gas bacillus (B. Welchii) which are the two most common causes of post-abortal sepsis.

The mechanisms of jaundice from different organisms are not uniform. That of Welch bacillus infection is the best understood (52). It is a fulminant hemoly-

tic anemia with hemoglobinuria, and with methemoglobinemia and hematinemia The same type of jaundice may occur in the rare septicemia caused by the anaerobic staphylococcus

Gas bacillus sepsis causes no jaundice in experimental animals and only in a small percentage of the clinical cases

The mechanism of jaundice from hemolytic streptococcus septicemia is not well understood At post-mortem examination most of the cases are found to have only diffuse cloudy swelling of the liver cells and the degree of degeneration bears no correlation with the degree of icterus (53)

A small distinctive group has been described by Babes (54) in which focal necrosis is found throughout the liver and streptococci are easily demonstrable in these areas There are no abscesses and the lobules do not show central necrosis It is possible to reproduce this lesion by injection of the organisms into the portal vein of experimental animals

Recently it has been claimed that jaundice may be a complication of bacillary dysentery In an institution in Vermont there were 67 cases of dysentery (Flexner) of which 35 developed jaundice Simultaneously, there were 54 cases of jaundice without dysentery Analysis of the time incidence seems to make it probable that there were two different epidemics, one of dysentery and the other of simple jaundice (Thorne and Estabrook (55a)) Brucellosis has been cited as a cause of hepatitis (55b)

Syphilis

Congenital Syphilis Liver involvement occurs in about one-half of the fatal cases of congenital syphilis (56) Many, but not all of these infants show jaundice Several different anatomic pictures occur (57) but the most characteristic is diffuse interstitial hepatitis in which huge numbers of spirochetes are found (58)

Icterus Syphilitic Precox The occurrence of jaundice in secondary syphilis has been recognized since the eighteenth century Its frequency is estimated to be between 0 37 and 1 4 per cent Ten per cent of the cases develop acute yellow atrophy The duration when untreated may be three weeks to three months In 25 of 30 cases seen by Lasch (61) the jaundice appeared two to four months after the appearance of the chancre It occurs generally at about the time of the first exanthem, but occasionally with one of the recurrent crops of skin lesions (60) (61) (62)

The clinical findings are indistinguishable from those of simple jaundice and differentiation rests on the presence of a chancre or secondary eruption (usually roseola) The serodiagnostic reaction may be positive or negative at that time An important point is the prompt clearing of the jaundice which is due to secondary syphilis when arsphenamines are given In fact, recurrence of jaundice has been noted, if treatment of secondary lues has been stopped too soon It is important not to confuse this disease, which occurs in untreated cases of syphilis, with paratherapeutic jaundice (see below)

The pathogenesis is disputed, but on the basis of its fairly frequent progression

into acute yellow atrophy, the view of Herxheimer that it is a parenchymatous degeneration, seems most acceptable. In fatal cases, the search for spirochetes in the liver has regularly been negative (59).

In the last ten years three of the cases of non-obstructive jaundice observed on the wards of The Mount Sinai Hospital were due to secondary lues. The following is a recent case:

The patient was a white man of 29 who gave a history of inadequate diet for several months, abdominal cramps for three weeks, and jaundice with clay-colored stools for two weeks before hospitalization. The clinical and laboratory findings were consistent with severe simple jaundice. The icteric index (acetone method) was 80, the cholesterol ester ratio was 350:120. His galactose tolerance test was 8.4 grams in 5 hours. The urine contained bile and tyrosin. The stools were positive for occult blood. The Wassermann reaction repeatedly was four plus. There was no history nor evidence at that time of a luetic mucocutaneous lesion.

The case was interpreted as one of severe simple jaundice perhaps precipitated by the malnutrition, and the serologic test was considered a false positive reaction. His jaundice cleared up almost completely by the sixth week after the onset and his general condition was much improved. A macular rash appeared at this time and was considered more probably toxic than luetic. Biopsy determined the syphilitic character of the skin lesion. Standard antiluetic therapy including arsphenamine was given and the rash disappeared several days later. His general condition remained good.

The other types of syphilitic hepatitis are gummatous or cirrhotic. The question of jaundice associated with antiluetic therapy and with hepatorecurrence will be discussed in the section on chemical jaundice.

Tuberculosis

Jaundice is far more common in tuberculosis than is generally considered and may occur with or without actual tuberculous lesions in the liver. When it occurs with tuberculous lesions, it is usually in rapidly progressive forms of the disease. The mechanism, as described by Ullom (63) and by Keefer (90), is heart failure or chronic anoxemia in 94 per cent and amyloidosis in 10 per cent of a series of cases which were examined post-mortem.

Warthin (64) studied several cases illustrating different types of actual tuberculous involvement of the liver. He classified these as follows:

1) Acute tuberculous focal necrosis of the liver.
2) Pylephlebogenous tuberculosis.
3) Bile duct tuberculosis.
4) Acute general miliary tuberculosis.
5) Terminal miliary tuberculosis of the liver in cases of chronic tuberculosis.

"It is particularly in the fulminant typhoidal types of tuberculosis that icterus is likely to occur, and the occurrence of jaundice in the early stages of affections showing a typhoid picture, but without certain diagnosis is more in favor of acute tuberculosis than of typhoid fever. Jaundice is rare in typhoid fever and when it does occur is found late in the course". (Warthin (64)).

Warthin cites in detail the case of one patient with jaundice, remittent chills and fever, whose condition was diagnosed as Weil's disease; another one in Georgia had been diagnosed as yellow fever. McCurdock, (65) in 1929, described

the case of a young woman with arrested pulmonary tuberculosis who developed what seemed to be a bout of acute catarrhal jaundice. A subsequent attack terminated fatally with the diagnosis of a possible suppurative pylephlebitis. Periportal tubercles were found disseminated throughout the liver.

The jaundice of tuberculosis is usually mild; however, in cases with gross anatomic liver involvement it may be intense (66). Except in the rare instance of obstructive jaundice due to tuberculous bile duct involvement, the icterus appears due to liver cell damage.

Acute Infectious Mononucleosis

The question of the jaundice complicating acute infectious mononucleosis (Pfeiffer's glandular fever 1889) is difficult. In fact, until the discovery of the heterophile reaction by Paul and Bonnell, there was question as to the clinical unity of the cases, and even now there is doubt as to the acceptability of the diagnosis of cases which show the clinical features but lack a positive heterophile test. Hematologists appear to regard as characteristic a minimum lymphocyte count of 50 per cent and a special type of "lymphocytoid lymphocyte". But the same type of cell is acknowledged to occur frequently in other conditions and we would be on firmer ground if we accepted as conclusive only those cases which show characteristic clinical features, blood picture and heterophile test.

On this rigid basis we have seen, in the past four years, two cases of jaundice among 67 cases diagnosed as infectious mononucleosis. Snapper, Eijkens, and Terwin (67) in 1922 reported a case of infectious mononucleosis with all the clinical features of "epidemic catarrhal jaundice". Heck (68) saw one case of jaundice complicating 44 of mononucleosis but MacKinley (70) saw five cases of jaundice in his series of 50. Mackay and Wakefield (69) in 1926 described a case with jaundice which they thought due to obstruction of the common bile duct by swollen lymph nodes. However, it is now generally accepted that the presence of simple inflammatory lymph nodes in the porta hepatis are not adequate to cause obstruction.

Jaundice complicating infectious mononucleosis is a very mild disease and in no way differs from simple jaundice. There are no reports as to the pathology of the liver and few reports as to liver function tests. Since the jaundice thus far has been mild and transitory and the clinical features suggest simple jaundice to the point of confusion, it seems valid to assume that the jaundice is due to hepatitis.

The question as to the identity of the two diseases may arise when in simple jaundice the blood picture shows a lymphocytosis with abnormal lymphocytes similar to those described in infectious mononucleosis (as has been described by Findlay and Dunlop in epidemics). The infectious agent of either disease is unknown; they both predominantly affect the younger age group; there are statements as to the occurrence of mononucleosis in families which implies that there may be an epidemic incidence.

Because of the rarity of proved cases of jaundice complicating mononucleosis we present the two instances which fulfill the rigid criteria set up above.

1) A white woman of 34 became ill with fever of 102°F., general lymphadenopathy, and slight hepatosplenomegaly. She developed jaundice after ten days of illness and the icteric index (acetone method, normal 3) was 13; the blood count showed 7600 white blood cells with polymorphonuclear leucocytes 27 per cent; lymphocytes 70 per cent of which 36 per cent were ordinary lymphocytes; and 34 per cent, lymphoidocyte type (Rosenthal). The heterophile test was positive 1:512 on admission, (at which time the temperature had risen to 104°F.), and later during convalescence, was positive in a dilution of 1:1024. The blood culture was sterile, the urine showed three plus bile, and urobilinogen at a dilution of 1:80, the galactose tolerance test was negative. The temperature slowly fell to normal and the jaundice and hepatosplenomegaly cleared up within two weeks.

2) A white woman of 28 became ill two weeks before admission with a shaking chill, fever, lymphadenopathy, and slight splenic enlargement. Jaundice was noted two days later and lasted one month. The icteric index rose to 9 (acetone method). The white blood count was 31,000 with 69 per cent lymphocytes (of which 19 per cent were lymphoid) and 3 per cent mononuclear cells. The heterophile reaction was positive, 1:256 and 1:580. The urine contained one plus bile and 1:80 urobilin.

Malaria

Malaria is frequently spoken of as predisposing to jaundice and probably jaundice does occur occasionally as a complication. Thus Wile and Sams (71) report it in 2 per cent of neuro-syphilitic patients treated by malaria infection. However, the characteristic and important form of jaundice complicating malaria is blackwater fever,—"a disease of disputed origin but undoubtedly associated with subtertian or aestivo-autumnal malarial infection, characterized by the occurrence of attacks of hemoglobinuria, caused by hemolysis or red blood cells in the general circulation and accompanied by jaundice and profound anemia" (72).

"Blackwater fever" is a disease of the tropics and a considerable length of residence in certain intensely malarial regions is necessary before the disease develops. The theory that the disease is due to a quinine idiosyncrasy or to an unknown infection accompanying malaria has been abandoned. The jaundice is undoubtedly due to an overwhelming release of bilirubin from the sudden destruction of as much as 60 to 80 per cent of the red blood corpuscles. This is shown by the fact that the liver parenchyma shows only minor changes at autopsy,—cloudy swelling of the cells which are filled with yellow pigment, congestion of the capillaries, and frequently areas of focal necrosis.

In addition, hypertrophic cirrhosis is asserted to be a not infrequent sequel of chronic malaria and this disease, of course, if often accompanied by jaundice. Oroya fever, occurring in Peru, is similar to blackwater fever.

On reviewing those infections in which hepatic infection is the dominant feature,—namely, simple jaundice, spirochetal jaundice, and yellow fever, (Group A—Chart I)—there is one striking common factor to which attention has not previously been drawn,—that the liver degeneration has the character of a second disease, in that the stage of infectivity usually ends as the jaundice appears. In all of these diseases permanent specific immunity occurs. In contrast (Group B) there are many infections in which jaundice is only a complication and may occur in various stages of the major infection.

Part II

JAUNDICE DUE TO CHEMICALS

Hepatic disease can be induced by a variety of chemicals both in industry and in chemotherapy. Sporadic cases of jaundice which might well be due to toxic agents but whose cause is seldom discovered are everyday occurrences. It is, therefore, important to have in mind all the known hepatotoxic agents and we present a list, which, though undoubtedly incomplete, contains all the chemicals to which we have been able to find reference. (Charts III and IV.) Before discussing the individual substances we shall consider some common factors influencing their action.

The mechanisms of the deleterious action of chemicals on the liver are only partly understood. To be considered are direct toxic effects, cumulative action, allergic sensitivity, non-allergic hypersusceptibility, and unknown causes termed "idiosyncrasy". We indicate below three major types of action. Some substances may have more than one.

First: Direct injury to liver parenchyma. Generally these substances (Chart IV) readily induce liver necrosis in animal experiments. Among them are phosphorus, chloroform, and carbon tetrachloride. Because of its excretory function the liver is especially exposed to the action of chemicals, such as these, which are general protoplasmic poisons (73). Among substances excreted by the liver are the dyes which are best known because their presence is so readily detected. The liver particularly excretes fat-soluble foreign substances, colloids, and also substances soluble only as basic salts, or in alkaline solution (74, 75, 76). The halogenated hydrocarbons are mostly fat-soluble and the phenolsulfonphthaleins are soluble only as basic salts.

Second: Primary hemolysis with secondary injury to the liver cells by the products of hemolysis. In addition to their direct action on red blood cells some of these substances may be directly injurious to the liver cells. In this group are phenylhydrazine, toluylenediamin, and hemolytic sera.

Third: Idiosyncrasy, hypersusceptibility, and allergic sensitivity in human beings. In experimental animals the injury is slight and not comparable to the human disease (77). This group includes some particularly important drugs, —the arsphenamines, sulfonamides, and cinchophens. It must be remembered that there are species differences in reactions to drugs and caution must be used in applying too literally the findings of one species to another.

Idiosyncrasy, according to Cushny, is an unusual effect of a drug for which no explanation can be found. The implication is that the individual possesses an unknown peculiarity of metabolism which gives him exceptional susceptibility. It may be that cumulative action, allergic sensitivity, non-specific (non-allergic) hypersensitivity, or some predisposing cause such as starvation with depletion of glycogen, protein or vitamins is also concerned with idiosyncrasy.

Cumulation (78) differs from anaphylactic shock and allergic sensitization in that its effects are identical with those obtainable by a single larger dose. They also differ in their mechanisms. The mechanism of cumulation may be chemical, as

CHART III

Chemical Grouping of Hepato-toxic Substances

A. *Inorganic Compounds:*
- Phosphorus
- Arsenic
- Antimony
- Manganese
- Copper
- Selenium
- Gold
- Bismuth
- Mercury

B. *Organic Compounds:*
- (Simple)
  - Alcohol
  - Ether
  - Divinyl ether
  - Carbon Disulphide
- (Halogenated)
  - Chloroform
  - Iodoform
  - Chloretone (Chlorbutanol)
  - Ethylchloride
  - Avertin (Tribromethanol)
  - Acetylene tetrachloride
  - Dichlormethane
  - Carbon tetrachloride
  - Monochlorethane
  - Monochlormethane
  - Monoiodoethane
  - Tetrachlorethane

C. *Aromatic Compounds:*
- Hydrazine
- Phenylhydrazine
- Toluylenediamine
- Picric Acid (trinitrophenol)
- Acriflavin (trypaflavin)
- Tetraiodophenolphthalein
- Diphenylchlorarsine
- Nitrobenzine
- Coal Tar (Shale oil)
- Dinitrophenol
- Guanidin compounds (Synthalin)
- Ieterogen (pyrrolarsanilate)
- Trinitrotoluol
- Arsphenamines
- Cinchophens
- Sulphonamides
- Alkaloid Amanitatoxin

D. *Biologic Substances:*
- Bean Poison
- Snake Poisons
- Hemolytic Sera
- Incompatible blood
- Liver Extract

E. *Physical Agents:*
- Distilled Water
- Burns

the result of the actual storage in the body of substances or of their derivatives; or it may be organic, due to changes in the body set up by the chemical and persisting after the substance has been eliminated.

CHART IV

Chemical Agents Producing Jaundice Arranged According to Type of Exposure and Predominant Mechanism of Action

	GROUP I DIRECT INJURY TO LIVER	GROUP II HEMOLYSIS	GROUP III IDIOSYNCRASY OF "ALLERGY"
Therapeutic (agents)	Gold Ether Chloroform Iodoform Avertin Tetraiodophenolphthalein Dinitrophenol Guanidin compounds "synthalin" Acriflavine Arsenic Carbon tetrachloride	Phenylhydrazine Sulfonamides Incompatible blood transfusions Trinitrophenol (picric acid) Hemolytic sera	Arsphenamines Cinchophens Liver extract Bismuth Mercury Sulfonamides
Accidental contacts	Amanitatoxin Bean poison Nitrobenzene Carbon tetrachloride Phosphorus Arsenic Burns	Snaks poisons Bean poison Incompatible blood transfusions	
Industrial hazards	Tetrachlorethane Carbon tetrachloride Nitrobenzene Manganese Carbon disulphide Trinitrotoluol	Arsine	
Experimental (agents)	Selenium Copper "Ieterogen"-pyrrol-arsanilate	Distilled water Toluylenediamine	

The facts about chloroform indicate the confusion that may arise between reactions due to direct injury to the liver cells and those attributed to an idiosyncrasy in human beings. Chloroform regularly produces liver damage in animals, but experience in the days when chloroform was a universally used anesthetic indicated that jaundice occurred only occasionally and this was therefore regarded as idiosyncrasy.

It is essential to bear in mind the criteria for acquired allergic sensitization which are as follows:

1) The causal agent or one of its immunologic relatives must have been encountered previous to the allergic reaction and may have been well-tolerated for a long time before.

2) Subsequent reactions differ qualitatively from the initial reaction and from the characteristic pharmacologic or toxicologic action of the drug.

3) Allergic reactions occur only in certain predisposed individuals and regardless of the deliberate increase in dosage can never be made to appear in the majority of individuals.

4) The allergenic substance may be inorganic as well as protein (79).

Non-allergic hypersensitivity may be due to a preceding injury and is not specifically acquired through previous exposure to the chemical which precipitated the untoward reactions. Thus even ether, which is harmless to the normal liver, may accelerate liver degeneration in the presence of previous hepatic injury. We have seen such a case in which ether administered to a patient who had recently had simple jaundice precipitated acute yellow atrophy. Infection may be synergistic with the toxicity of a drug,—as is illustrated in Opie's experiments (80) with colon bacilli and chloroform. The previous state of nutrition and vitamin storage undoubtedly is important as a predisposing factor, especially in the jaundice complicating the fatty liver of alcoholics, and in experimental carbon tetrachloride poisoning.

Many chemicals may injure other tissues and organs besides the liver. Thus, arsphenamine, trinitrotoluol and tetrachlorethane may produce dermatitis, toxic gastritis, aplastic anemia. Determination of the type of extrahepatic involvement may be of diagnostic help in obscure cases.

The following section is concerned with the systematic consideration of some of the substances in the classification.

Chemicals with Direct Action on the Liver

Arsenates (and arsenites) are of practical importance, not so much in acute poisoning in which the liver damage is slight, but in the more frequent insidious chronic poisoning. This may be due to insecticides affecting workers or food consumers; to metal ores; to pigments, preservatives; or rarely to prolonged use of Fowler's solution (potassium arsenite).

Recently von Glahn, et al. (81) have demonstrated focal liver necroses in rabbits as early as three days after the administration of insecticides.

Chronic poisoning (Cushny) may show coryza, conjunctivitis, jaundice, swelling of the liver, a variety of skin eruptions and pigmentations, and finally both sensory and motor neuritis or even encephalitis.

Arsine and the organic arsenicals are discussed under other captions.

Phosphorus: Acute phosphorus poisoning was common in the nineteenth century when phosphorus was used as an abortifacient and as a suicidal agent. It produces an extreme fatty degeneration of the liver and to some extent of other organs. It has been shown in experimental poisoning that the liver damage is

initial and the renal injury is terminal (Marshall and Rowntree (82)). Clinically there is intense jaundice with a marked hemorrhagic tendency, abdominal pain and collapse (Cushny). In those patients who live several days, acute yellow atrophy develops. Rare cases which survive develop coarse nodular cirrhosis. Chronic phosphorus poisoning, formerly important in industry, (83) does not affect the liver.

Manganese is of interest in the problem of Wilson's disease because experimentally it produces hepatic degeneration and cirrhosis and also degeneration in the brain. In industry neurologic symptoms have occasionally occurred following prolonged exposure to manganese (84).

Gold, as the thiosulfate etc., in the commercial products Crisalbine, Solganal, Myocrisin, Lopion, has been used empirically in the treatment of rheumatoid arthritis (85) and of lupus erythematosus with a variety of toxic side-actions similar to the action of mercury (86). These complications are generally an ulcerative gastroenteritis, nephritis, or blood changes such as purpura, agranulocytosis, or hyperkeratosis. The incidence of toxic actions is about 25 per cent of cases treated. Of these, jaundice and hepatic damage are among the less frequent and occur as a delayed action. Hartfall and Garland (87) cited eight mild cases of jaundice among 100 cases of rheumatoid arthritis treated with gold. The jaundice lasted three to four weeks and left no clinical evidence of hepatic damage. "The clinical picture was almost indistinguishable from the hepatitis which passes for 'catarrhal jaundice' ".

Anderson and Palmer, in reporting a fatality (88) in a patient treated with gold who developed ulcerative enteritis, describe the liver as showing necrosis of parenchymal cells around the central veins and slight fatty changes and granulation in peripheral cells. Jaundice had not been present. Freyberg (89) states that their animal experiments showed that the reticulo-endothelial cells of the liver and spleen take up gold when given in colloidal form.

There is no distinct correlation of toxic symptoms with dosage. The onset in 25 per cent of the cases is considered due to cumulation. As with cinchophen, the toxic symptoms may appear and progress to a fatal termination after the drug has been discontinued.

Antimony: Keefer (90) states that the treatment of granuloma inguinale and of kala azar with antimony compounds is occasionally followed by toxic jaundice.

Selenium: Studies on chronic selenosis (91) suggest that this condition, which occurs in lower animals, is a potential human hazard, though as yet no cases have been noted in man. Selenium occurs in the soil of South Dakota and thus is a contaminant of cereals and plants which cause "alkali disease" in lower animals. It is also used in insecticide sprays in the western United States (92).

In rabbits and cats which ingested selenium—containing foods spontaneously (dose from 0.2 mg. to 1 mg. per kg.) the liver showed mild to moderate chronic interstitial hepatitis, occasionally portal cirrhosis, and nodular cirrhosis. Young rats are more susceptible, with the occurrence of ascites, pleural effusion, advanced nodular cirrhosis and severe anemia.

Alcohol: We have frequently seen jaundice precipitated in chronically alcoholic, avitaminotic individuals by an alcoholic spree such as never produces jaundice in normal persons (93). Although this is a commonplace of clinical practice, we have found very little discussion of it. Conner (94) points out that fatty infiltration of the liver may develop very rapidly and that an enlarged tense fatty liver may actually cause intrahepatic block with jaundice and even portal obstruction and ascites. At autopsy the fatty liver may be the only finding. In more advanced cases, degeneration and atrophy at the periphery of the lobules, proliferation of connective tissue and finally typical portal cirrhosis occur.

Experimentally, William H. Welch (95) produced fatty livers with alcohol in rabbits and MacNider (96) in dogs.

Chloroform has long been known to induce liver damage and a hemorrhagic tendency, as well as general parenchymal destruction of many other organs. Jaundice, even acute yellow atrophy, was a frequent occurrence, thirty years ago, when chloroform was in widespread use (78, 97). The symptoms occurred ten hours to six days after anesthesia. Involvement of the liver was similar to that of phosphorus poisoning. However, characteristically the fat infiltration is less with chloroform (Wells (98)).

Whipple and Sperry (99) experimentally produced extensive central cell liver atrophy, which could be recognized microscopically about six hours after administration. They demonstrated that repair of the severely damaged liver was discernible in twenty-four hours and was complete in three weeks. Cirrhosis did not occur. Howland and Richards (101) were able to produce delayed chloroform poisoning with liver necrosis twenty-eight hours after anesthesia. The animals varied in their susceptibility to poisoning. H. L. Wolff (78), in experiments concerned with "cumulation" of drugs, demonstrated that when mice are exposed to chloroform for one-half hour, the maximum damage to the liver is not seen for six days. This damage consists not merely in degenerative fatty infiltration, but also in definite cellular injury. With repeated poisoning the fatty infiltration is pushed into the background by the cellular damage.

Recently, chloroform, in contrast to ether and cyclopropane (100), has been shown to damage the prothrombin-producing function of the liver even when no jaundice was produced (102). This may account for the hemorrhagic tendency.

Delayed chloroform poisoning has not entirely disappeared, as indicated by a recent (1040) study of its pathology by H. L. Sheehan (103). He encountered fourteen cases in a series of 400 obstetrical autopsies in England. He emphasized the distinction between chloroform poisoning and "obstetrical acute yellow atrophy." In the former true cell necrosis occurs such as is seen in animal experiments and in idiopathic acute yellow atrophy. "Obstetrical acute yellow atrophy" is pathologically distinctive and is not rare—6 among his 400 autopsies. It becomes manifest at the thirty-sixth or fortieth week of pregnancy and is fatal in one to two weeks. It is characterized by gross fatty change of the entire lobule except a narrow rim of normal cells. There is an entire absence of real liver necrosis; the lobular pattern remains intact.

Chloroform poisoning occurs principally in those obstetrical patients who have

had a long, exhausting labor with semi-starvation and occasionally with infections. Sheehan believes that under normal circumstances, chloroform is safe.

Avertin: The incidence of clinical liver damage due to avertin is evidently small. Henschen (104), who collected five instances of hepatic deaths and three cases of slight icterus, following avertin narcosis, found one series of 1306 such anesthesias without any harmful effects. We have seen one case of moderate jaundice undoubtedly following avertin anesthesia for herniotomy.

Because of the chemical similarity to chloroform, direct action on the liver parenchyma may be presumed, but it is possible that the severe anoxia which often occurs with avertin may play a role.

Carbon Tetrachloride is widely used in industry as a solvent for gums and fats, in rubber cements, in fire-extinguishers (105), and in household cleaning (Carbona). It is used as a vermifuge and occasional fatalities have occurred. Its toxic action resembles that of chloroform, but is generally said to be slower and less reversible. However, the outstanding effects in industry are as an irritant of the mucous membranes and of the central nervous system.

Another type of clinical picture was reported by Garin (106) et al., in 1938. They describe the occurrence of "hepatonephritis" in two persons several hours after the inhalation of fumes of carbon tetrachloride. The onset was sudden with vomiting, severe headache, transient fever. The urine was much diminished and contained traces of albumin and of urobilin but no bilirubin. There were transient jaundice, occasional epistaxes, ecchymoses and nervous disturbances. The liver varied in size and in tenderness. Azotemia was present. Recovery occurred in three weeks. In spite of the severity of the clinical picture the authors give a good prognosis.

Experimentally carbon tetrachloride causes severe necrosis of the liver. It has been used to produce experimental cirrhosis of the liver in guinea pigs (107, 108). It is stated that recovery from previous exposure endows the animals with increased resistance to injury from this chemical and on this basis a routine of increasing slight exposures has been proposed to diminish industrial hazards.

Acriflavine: In 1930 it was reported that 11 per cent of English soldiers treated for gonorrhea with intravenous injection of acriflavine developed symptoms indistinguishable from epidemic catarrhal jaundice eight or more weeks after cessation of treatment (109). There was one case of acute yellow atrophy. In untreated troops, the incidence of jaundice was only 0.32 per cent. The author stated that "the occurrence of jaundice in these cases must be attributed to some form of idiosyncrasy which was present" in about 11 per cent of the cases treated.

In 1933 Joanissian (110) surveyed the therapeutic and toxic effects of acriflavine and cited the regular experimental production of hepatitis in guinea pigs and of nephritis in dogs. All indications are that acriflavine is a general protoplasmic poison rather than a substance whose toxic effect depends on "idiosyncracy."

Dinitrophenol, which had a brief popularity as an obesity cure, was known as a poison in the First World War. Warthin (111), in 1918, reported the first

study of the liver and found acute hepatic degeneration similar to that from chloroform or trinitrotoluol. The therapeutic use has been complicated by clinical liver damage in about ten cases reported in recent years.

One patient who received approximately 360 mg. of dinitrophenol for two weeks developed urticaria and pruritus followed in five days by intense jaundice, an enlarged tender liver, epigastric cramps, clay-colored stools, and albuminuria. There was gradual improvement after seven weeks (Sidel (112)).

Experimentally, it has been shown that dinitrophenol, as might be expected from its acceleration of metabolism, produces rapid depletion of liver glycogen. Liver function was studied in eight patients receiving therapeutic doses of dinitrophenol. In six there was increased bromsulphalein retention without any significant change in the other standard tests.

Synthalin, introduced about 1930 in the treatment of diabetes because of its property of lowering blood sugar, proved to be a general liver poison. This was shown by the loss of the deaminizing power, glycogen storage by the liver and by the occurrence of acute yellow atrophy (Bischoff and Long (114)).

Tetrachlorethane (115), extensively employed in the First World War, in aeroplane varnish, was responsible for many cases of acute yellow atrophy. The portal of entry was via skin and lungs (116, 117). Its use was therefore discontinued (118).

Trinitrotolulol, used in munitions manufacture, causes either aplastic anemia or acute yellow atrophy. During the First World War, there were fifty cases of jaundice in England, occurring among many thousands of workers (119). The jaundice generally appeared after five to sixteen weeks of employment (Livingstone, Learmonth and Cunningham (120, 121)).

Icterogen: This pyrrol compound, pyrrolarsanilate, synthesized by Ehrlich, regularly produced fatal icterus in mice, rats and guinea pigs. Goldman produced experimental cirrhosis with this drug. Ogata noted that repeated administration of moderate doses developed tolerance in laboratory animals.

Ehrlich postulated that since liver cells metabolize bilirubin, urobilin, and other pyrrol compounds, they have chemoreceptors for these compounds, causing the pyrrolarsanilate to be anchored in the liver with concentration of its toxic effect there (122).

Amanitatoxin (125): Cases of mushroom poisoning are not infrequent in country districts and usually involve groups of persons (123). They occur less often in this country than in Europe though the poisonous fungi are very common here. Nearly all are of the genus Amanita. Kobert, in 1891, discovered a powerful hemolysin which is thermolabile and therefore seldom important clinically. Abel and Ford (1906 to 1914) discovered a thermostable alkaloid, amanitatoxin, which is the active poison (126). Among other manifestations of liver damage this produces marked hypoglycemia in dog experiments.

The degrees of intoxication vary widely, but the sequence of events is uniform (126). The first symptoms, a violent gastroenteritis simulating cholera, appear eight to sixteen hours after ingestion. If the patient survives this, there is a two to four day quiescent period during which the liver becomes swollen. A

second toxic phase follows, characterized by severe and varied nervous manifestations and by symptoms due to hepatic degeneration, jaundice and hypoglycemia. The mortality is extremely high. At post-mortem examination, necrosis of the liver sparing only the periphery of the lobule is found, together with infiltration of the kidneys, heart, and nervous system, similar to the lesions of phosphorus poisoning.

One patient, with acute yellow atrophy due to amanitatoxin, died years later and was found to have a normal liver, but most cases that survive an acute attack develop nodular sclerosis (124).

Bean Poison: Bean poison is a common form of poisoning in animals, but only one variety, favism, is recognized in human beings (MacCrae (127)). Favism is an acute hemolysis (Group II of Chart IV) due to ingestion of a special variety of bean (vicia fava) or to inhalation of its pollen. It occurs chiefly in southern Italy, but has been seen in America (MacCrae (127)). After an incubation period of 1 to 48 hours there is a sudden intense hemoglobinuria, jaundice and anemia followed by enlargement of the liver and spleen.

The mortality in Sardinia is given as 8 per cent.

There are several varieties of bean poisoning in animals. One is the Senecio disease of South Africa and Australia (Roman (27)). This is an acute yellow atrophy in horses and cattle due to ingestion of the senecio weed.

The more widespread lupinosis of sheep, goats, and cattle is similar, and occurs after contamination of the fodder by wild lupines. This is also an acute yellow atrophy and some animals which recover develop cirrhosis (Hutyra, Marek and Manninge, Luisada (128)).

Diethylstilbesterol has been accused of being a cause of liver necrosis but the most recent evidence is that the apparent liver necrosis is a cellular vacuolization due to the deposition of large amounts of glycogen. Clinically the toxic symptoms are gastro-intestinal (129).

PHYSICAL AGENTS CAUSING HEPATIC DEGENERATION

Burns

It is not generally recognized that liver damage is part of the toxemia of severe burns. Bardeen (130) in 1898 described merely cloudy degeneration of the liver as a minor complication of burns. The subject received little recognition until 1938 when Wilson, MacGregor, and Stewart (131) studied 65 cases of severe burns with 20 necropsy examinations. Twelve cases had jaundice. Some cases without jaundice, nevertheless, showed liver damage on post-mortem examination. The jaundice occurred as early as 48 hours after the burn and was most pronounced in the slowly progressive low-grade toxemia of adults, appearing about the fourth day. The authors point out that there was no correlation with the type of treatment or with the occurrence of sepsis. The severity of the liver lesion was, however, closely correlated with the degree of toxemia.

In the first twenty-four hours after the injury there is fatty degeneration about the central veins. Subsequently, there is necrosis of hepatic cells and hemor-

rhages in the sinusoids giving a nutmeg appearance. In two cases, extensive eosinophilic infiltrations, almost entirely restricted to the liver, were noted.

The authors regard liver necrosis and degeneration as the most striking and characteristic feature of the pathology of burns. The observations of these authors were confirmed not only by subsequent human studies, but also by animal experiments of burns immediately treated with tannic acid.

The pathogenesis of liver injury has been attributed to a non-bacterial toxin from the necrotic areas or to anoxia due to shock, plasma loss and hemo-concentration (132, 134, 135).

In 1940 we saw a patient with extensive second and third degree burns which were immediately treated with tannic acid spray followed by local application of gentian violet. She had a septic type of fever throughout the clinical course. On the third day she developed jaundice which became progressively worse. She died in two weeks with the clinical picture of acute yellow atrophy. At that time we assumed that the jaundice was due to the burn. However, it is striking that all the observations on liver necrosis have been made during the period in which tannic acid has been the routine treatment. Even in the confirmatory animal experiments tannic acid was applied at once (133). Therefore, while we believe that the toxic effect of the burns proper is the most probable explanation, the possibility of a toxic effect of tannic acid will have to be ruled out by further experiments.

CHEMICALS WITH HEMOLYTIC ACTION

The substances in the second group (Chart IV) are predominantly hemolytic. It has been difficult occasionally to state whether the hepatic degeneration is dependent on the hemolysis or due to a direct action on the liver.

Relation of hemolysis to the liver: While a discussion of hemolytic jaundice as such is beyond the scope of this paper, it should be mentioned that every condition producing rapid and extensive intravascular hemolysis causes jaundice; for example, malaria, hemolytic serum, incompatible transfusion. The liver is the organ which normally disposes of the products of hemolysis. That the jaundice is due to an overburdening of the liver rather than to acute liver degeneration by primary injury is shown by 1) the enormous masses of hemoglobin liberated in these diseases (Fox and Ottenberg (137)), 500 to 700 grams, as compared with the normal capacity of the liver of excreting the bilirubin derived from 12 grams of hemoglobin per day; 2) the rapid subsidence of the jaundice in those cases which recover; 3) the presence of only minor lesions in the parenchyma of the liver in the fatalities.

For the above reasons, it may be stated that when a chemical known to have hemolytic properties causes jaundice with marked liver necrosis, the liver lesions are to be attributed to a primary action of the drug rather than to the hemolysis.

Of the products of hemolysis, hemoglobin itself is only slightly toxic and is rapidly converted into bilirubin which is harmless; but the cell stroma has been shown to be toxic. Because of the insolubility of the stroma there is much phag-

ocytic activity by the Kupffer cells. The high lipoid content of the stroma suggests that the degradation products may be especially excreted by the liver parenchyma.

Mild degrees of hemolysis may produce "retention" jaundice (Rich (47)) with large amounts of bilirubin giving only the indirect Van den Bergh reaction of the blood and a great excess of urobilinogen in the urine as well as in the stool. The urobilinuria is characteristic of all forms of hemolytic jaundice. In other forms of jaundice, urobilinuria is present only in the recovery phase.

Arsine (Hydrogen arsenide) is a dangerous gas liberated in many major industries. It has essentially a hemolytic action producing hemoglobinuria, jaundice, acute hepatitis, and acute nephritis. Mass poisoning has occurred in submarines in the First World War from storage batteries (138).

The *Phenylhydrazin* group used in the treatment of polycythemia vera frequently causes jaundice, hepatosplenomegaly, abdominal distress, nausea, anorexia, and marked weakness corresponding to the period of rapid hemolysis. In industry, the hydrazine group is associated with a variety of symptoms and lesions,—pruritus, eczema, cirrhosis of the liver; degenerative changes in the liver, spleen, and spinal cord,—the last resembling those of pernicious anemia. The destruction of red blood cells by some of the hydrazine group is followed by only a minor increase in serum bilirubin, which is in contrast to toluylenediamine. The anemia-effect and the organotoxic action do not always run parallel. Hooper (139) studied the action of this group in experiments on rats. He did not find any significant organic lesions in those animals dying two to eight hours after the injection. Severe parenchymatous degeneration and massive vascular damage occurred in those which died later.

Toluylenediamine produces a hemolytic type of anemia predominantly in cats, and icterus predominantly in dogs. Large doses in dogs also result in hemoglobinuria. The anemia precedes the jaundice. Hemolysis is diminished in splenectomized animals and is absent in vitro. The mechanism of this type of jaundice has been the object of many experiments and there still is controversy as to the relative importance of the disturbed function of the liver parenchyma and of the inflammation of the fine bile ducts (140).

The *Saponin* group, containing antiquated drugs now unimportant, includes sarsparilla, quillaja, solanin and senega. All of the members of this group are hemolytic poisons even in vitro and have been responsible for clinical poisoning in the past.

It has been known for centuries that the *transfusion of incompatible blood* frequently causes jaundice (141). While the jaundice is due in the first place to functional overburdening of the liver, fatal cases show parenchymal damage consisting of foci of necrosis in the central cells with associated hemosiderosis of Kupffer cells. Agglutinated red blood cells become impacted in the hepatic sinusoids and form hyaline thrombi. The lesion has been compared to that occurring in eclampsia.

Clinically, though jaundice occurs promptly, it is unimportant. The outstanding symptoms are those associated with renal rather than with hepatic damage.

Hemolysis may occur on a *physicochemical* basis because of changes in osmotic tension. Thus, distilled water, intravenously injected, may cause hemoglobinuria, albuminuria, and jaundice, depending upon the rate and quantity of flow. Krumbhaar (142) found in experiments on himself that doses which just fail to cause hemoglobinuria are followed by the appearance of bile and albumin in the urine that day. The extent of anatomic liver damage has not been determined.

CHEMICALS AND HUMAN IDIOSYNCRASY

The mechanisms of action of the members of the third group (Group III, Chart IV) are the least understood and the most disputed.

Arsphenamines: Jaundice may follow the use of any of the arsphenamines (143). A distinction is drawn between the occurrence of the *early* type within the first week or two after an injection, and of the *delayed* type which may occur as late as three to ten months after termination of a course of treatment.

The *early* cases are directly associated with the injection in point of time; most of them show initial toxic symptoms immediately after the injection, fever, chills, gastro-intestinal disturbances, toxic erythema. Overdosage does not play a role. The syndrome "erythema of the ninth day" (144), characterized by a morbiliform or scarlatiniform eruption, fever, and general toxic symptoms, which occurs occasionally after arsphenamine therapy, may precede the onset of jaundice. It has been stated that it is safe to continue the arsphenamine therapy after the subsidence of this rash if jaundice has not appeared. But Robinson has reported that several cases, in which this was done, developed hepatitis and jaundice, purpura, or nephritis.

The *delayed* type of icterus is the more frequent form (about two-thirds of all cases). Not all authorities are agreed that it is actually due to the drug. The clinical picture may vary from that of "simple jaundice" to acute yellow atrophy. Liver functions were studied routinely in twenty-five patients receiving massive dose chemotherapy for early syphilis by the intravenous drip method (145). Four patients developed mild jaundice without permanent damage. In twenty-two other patients there was a transient rise in the icteric index (above 10-15, acetone method). None of these developed hepatitis of clinical note.

Bilirubin excretion tests on ten consecutive unselected patients showed slight impairment of function in three. The authors emphasize the absence of clinically demonstrable hepatic degeneration and atrophy in these patients.

In recent years it has become evident that there are two types of jaundice following the use of the salvarsans. The more common shows the laboratory findings typical of hepatocellular damage. The less frequent type is characterized by laboratory findings of biliary obstruction such as elevated blood cho-

lesterol and cholesterol ester, high blood phosphatase, and negative cephalin flocculation test (146) On biopsy these cases show "bile thrombi" in the biliary canaliculi with cholangiolitis similar to that described by Naunyn (146)

The first type is more frequent in the delayed salvarsan jaundice, and the obstructive type is seen particularly in early salvarsan jaundice

The point of view that the jaundice is dependent on the arsphenamine is based on a number of well recognized facts·

1) Definite anatomic syphilis of the liver is rarely associated with jaundice

2) Arsphenamine jaundice is not accompanied by serologic evidence of recurrence

3) Experimentally, arsphenamine does damage the liver functions and large doses can produce jaundice in dogs (147)

4) The increased incidence of jaundice is definitely paratherapeutic Ruge (15) found that luetic sailors treated with arsphenamine had jaundice eight times as frequently as a similar non-luetic group One of Sager's (148) patients had two attacks of paratherapeutic jaundice ten years apart

5) Paratherapeutic jaundice is also observed as a complication in non-syphilitic cases Ruge had fifty-two such cases in his series, Wile and Sams (71) had three We have recently observed a patient with subacute liver atrophy apparently precipitated by arsphenamine therapy for Vincent's angina

6) There is occasionally concomitant occurrence of other symptoms of arsphenamine poisoning such as the characteristic dermatitis or the aplastic anemia

7) Liver damage has been demonstrated in the latent period of delayed arsphenamine jaundice (reminiscent of Bergstrand's observation of the picricterie stage in the epidemic liver atrophies) A similar variable latent period has been demonstrated with other hepatotoxins, for example, chloroform

An alternative explanation is that delayed arsphenamine jaundice is really due to the infectious agents of simple jaundice and that arsphenamine acts only as a superimposed factor Thus Stokes, Ruedemann and Lemon (149) in 1920 showed that cases of arsphenamine jaundice seem to occur in groups and particularly at times when simple jaundice is prevalent in the community Todd (150) showed that arsphenamine jaundice, like simple jaundice, is more common in winter Studies in the German navy showed that the incidence of simple jaundice and of arsphenamine jaundice was roughly parallel over a period of ten years

In a military hospital at Cambridge, England, 2933 patients were treated with arsphenamine in 1918 Of these 37 developed jaundice within seven months Fifteen of these died with acute yellow atrophy At the same time there was an epidemic of simple jaundice among children of that district (151)

There is a third theory expounded by Mihan (152), that the delayed jaundice of arsphenamine therapy is really due to a hepatic recurrence of syphilis and not to arsphenamine He bases this idea on the fact that the nervous or mucocutaneous recurrences of syphilis occur at about the same time interval as paratherapeutic jaundice Mihan continued to treat 75 cases with arsphenamine

and claimed that the results were satisfactory in 60, although 11 were intolerant to treatment and 4 died (which he did not emphasize). Other workers have not confirmed his claims. Ravant, using Milian's method, had two fatalities (148).

We have seen a patient with clinically latent syphilis treated with arsphenamine in spite of two intercurrent attacks of jaundice, who finally developed hepatosplenomegaly and aplastic anemia.

Differential diagnosis between simple jaundice and arsphenamine jaundice may be difficult. An asymptomatic onset, or diarrhea and dermatitis are more frequent in arsphenamine jaundice, while abdominal pain and tenderness are more frequent in simple jaundice.

We believe that arsphenamine is of prime importance in most cases of delayed arsphenamine jaundice. In addition, the occurrence of arsphenamine jaundice with the epidemics of simple jaundice indicates that in some patients the two factors may be synergistic. It must be acknowledged that occasionally simple jaundice *per se* may develop in a patient who incidentally is receiving antiluetic therapy.

An estimate of the actual danger from the use of arsphenamines is of practical importance. In Wile and Sams' (71) carefully treated series of 4,126 cases there were 56 with hepatitis and 1 death. Ruge (15) gives the mortality of epidemic jaundice as 1.24 per cent (8 cases among 647). There was one fatality directly due to arsphenamine hepatitis in our much smaller series of 12 cases of arsphenamine hepatitis.

Soffer (143), in a study of 18,250 luetic patients at Johns Hopkins Hospital, reported an incidence of 81 cases of arsphenamine jaundice. These patients were subsequently treated again with arsphenamine, and two had a recurrence immediately after the first injection. The duration of jaundice showed wide variation, from 10 to 100 days. Six per cent of the jaundiced patients developed acute yellow atrophy. Ten per cent had an associated arsphenamine dermatitis.

He found that arsphenamine causes jaundice 50 per cent more frequently than does neoarsphenamine, and tryparsamide is the least icterogenic. Mapharsen has also been found to be icterogenic.

Bismuth and Mercury: Bismuth and mercury also, but far less frequently than arsphenamine, are hepatotoxic agents. Nomland et al. (153) report 32 cases of jaundice believed to be due to the injection of bismuth subsalicylate. Of these 22 had also received neoarsphenamine but none within twelve weeks of the beginning of jaundice. Ten of the patients had had bismuth only, and these may be regarded as unimpeachable evidence. All of the cases recovered. Most were given bismuth again without subsequent harm. The incidence of bismuth jaundice was one attack for 2242 injections and of neoarsphenamine jaundice one for 951 injections.

Wolman (154) reports the death of two infants from acute yellow atrophy following intramuscular injection of sodium bismuth thioglycolate. Both were apparently normal infants with no clinical manifestations of syphilis, one received only one dose of the drug and died four days later.

Syphilis has been considered the essential etiologic agent for jaundice in cases

treated with bismuth and mercury. However, an occasional non-luetic patient has been treated with mercury with the complication of liver damage. Tileston (155) reported a case of acute yellow atrophy in a nine year old boy who received mercury inunctions for two months, although he was not luetic. Aside from these few cases there is neither clinical nor experimental evidence that mercury is a liver poison.

Although *Cinchophen Jaundice* is frequently regarded as an allergic manifestation, Brugsch and Horsters (156) demonstrated that the cinchophen group affects the liver cells. They found that the immediate effect after intravenous injection in dogs was an increase in the amount of bile. This was attributed to direct stimulation of the liver cells. They even suggested that the flow of bile in response to atophan administration be used as a differential test between obstructive and non-obstructive jaundice. On the basis of their research Grunenberg and Ullman used cinchophen clinically both in patients with normal livers and with "catarrhal jaundice", confirmed the choleretic action, and claimed beneficial results.

Jaundice following the clinical use of cinchophen was first described by Worster-Drought in 1923 and has since been reported with increasing frequency. A few still believe that the role of cinchophen has been exaggerated. The reported mortality is high, about 50 per cent of 191 cases in 1936. The real mortality is undoubtedly not as high though the morbidity must be much higher. The symptoms are similar to those of other forms of hepatitis, but for the greater clinical severity and the higher mortality.

As with arsphenamine, the jaundice may appear as long as two months after the cessation of the use of cinchophen. Likewise the incidence and severity of the jaundice bear no relation to the size of the dose and there are numerous individuals who have taken enormous doses without any symptoms. In several fatalities the dose of cinchophen has ranged from 37½ grains to 2700 grains with intervals of one to sixty days between the time the drug was discontinued and the time of onset of toxic symptoms. Occasionally jaundice has occurred after cinchophen has been taken continuously, stopped, and a small dose resumed. Rabinowich cites one case in which two attacks of jaundice followed two courses of cinchophen therapy. Palmer and Woodall (157) believe that there is no safe method of dosage, because toxic symptoms may occur with small doses, and even when the drug is stopped at the very onset of symptoms, a fatal outcome may not be averted.

Most of the fatalities occur within the first six weeks of jaundice, 52 per cent of the deaths occurred in the first two weeks. Ascites was noted in thirteen of 117 cases; in only 2 did this finding appear in the first three weeks of illness (158).

Although jaundice has been tacitly accepted as the indicator of liver damage in cinchophen administration, Weir and Comfort (159) describe four cases of hepatic injury without clinical jaundice. One had urobilinuria of thirty days duration, another had an enlarged liver and dye retention; a third had subclinical jaundice and peculiar abdominal symptoms. In two cases described by Quick (160), symptoms and signs of liver disease were present without jaundice.

Experimentally, the production of jaundice by cinchophen has been uncertain. Barbour and his co-workers were able to induce jaundice in one series of rats and not in another. Jaundice has not been produced in dogs by means of cinchophen.

A number of authors (Quick, Reichle and others (161)) have suggested that cinchophen jaundice is due to sensitization. As confirming this, they point to the supposed allergic symptoms such as skin eruptions, edema, vasomotor disturbances.

Of this group of drugs neocinchophen (tolysin) seems to be far less toxic both clinically and experimentally, while the iodocinchophens and the salicylate compound, atophanyl, seem to be more dangerous than cinchophen.

One must conclude that the sequence of cinchophen therapy followed by jaundice or acute yellow atrophy is too frequent to be dismissed as only a coincidence.

Sulfonamides: Jaundice complicating treatment with the newer chemotherapeutic agents,—sulfanilamide, sulfapyridine, and sulfathiazole, is fairly common, more so than with the arsphenamines or cinchophens. Watson and Spink (162) saw 16 cases in their large series and estimated about 1 case in 150 treated. Long, Bliss and Finestone (163) report two cases among 408 treated with sulfanilamide. We have seen over 20 cases ourselves. Four of these cases have been described (163). As far as is known at present the three sulfonamides are about equally prone to cause jaundice, but it may occur with any of the related drugs including prontosil.

The jaundice occurs as a result of the following mechanisms: 1) hemolytic anemia; 2) hepatic degeneration (most frequently); 3) rarely, a combination of the two preceding types.

1) Acute hemolytic (hemoglobinuric) anemia from sulfonamide compounds is a relatively rare but distinctive disease. With great regularity it comes on within four days from the beginning of drug administration. It sometimes occurs after very small doses—we have seen it follow as little as 4 gm. in one day. There are no prodromata. The patient suddenly complains of severe general pains, especially of deep lumbar pain. He may be nauseated and occasionally vomits blood. The fall in hemoglobin occurs within several hours and may be as extensive as from 100 per cent to 30 per cent in twenty-four hours. The worst cases present the picture of severe shock. About 50 per cent of the cases recover. Death occurs within the first three days. In those who survive the initial shock, uremia may occur because of kidney blockage by hemoglobin casts.

The hemoglobinuria may be so slight as only to be detected on search or may be of the greatest intensity with urine of deep port wine color. Bilirubin is found in the urine when searched for, after the hemoglobin has been precipitated out.

Jaundice appears within a few hours after the onset of the hemoglobinuria. It is generally slight enough to be overlooked, but may be intense.

The stools are dark in color and may be hemorrhagic.

In fatal cases, the liver has shown no significant lesion. The patients have been too prostrate for the performance of liver function tests. The jaundice is

not indicative of liver damage, but of the overburdening of the excretory function of the liver by the tremendous amount of liberated hemoglobin.

2) On the other hand, the most common type of jaundice, that due to liver injury alone, does not appear promptly after a small dose as does acute hemolytic anemia, but generally after the drug has been taken for a week or two. Also it has appeared a week or more after the chemotherapy had been terminated. Greene and Hotz (164) report its occurrence one month, Bannick, Brown and Foster (165), ten days; Ottenberg (163), five and seven days, after termination of treatment.

It occasionally comes on after only a small total dose—in one of our cases after only 9 grams, but more often after a considerable amount. We have seen one case in which jaundice developed five days postoperatively following deposition of a single dose of 10 grams of sulfanilamide intraperitoneally in a patient with intestinal resection.

The onset of jaundice is insidious; the symptoms and course are similar to those of simple jaundice. The liver may be enlarged. The patients recover if the drug is stopped. Persistence of chemotherapy is dangerous (167).

Function tests, the bromsulphalein and the galactose tolerance test, were positive in most of our cases and in most of the reported cases. The blood cholesterol and cholesterol esters were depressed.

Some of the cases that started with mild jaundice died with acute yellow atrophy (169). Cline's (168) case showed also nephrosis. Occasional cases with acute yellow atrophy develop nodular sclerosis. Garvin's (170) case had developed ascites but recovered. Fitzgibbon and Silver (171) described a fatal case in a patient in whom treatment with sulfanilamide had been discontinued because of the development of a maculo-papular skin eruption. Two months later the patient, suspecting a recurrence of gonorrhea, took one gram of the drug. Within five hours he developed an intense skin eruption and fever, and five days later severe jaundice which lasted for a month.

When the associated dermatitis is exfoliative, the cases of sulfanilamide poisoning are indistinguishable clinically from arsphenamine poisoning (172).

Previous liver damage may predispose to acute hepatitis following sulfonamide therapy. Jaundice is more common in toxic patients. Hageman and Blake (166) have seen several cases develop in alcoholics and we have seen one such case. On the other hand, clinical experience shows that there is no particular danger of causing sulfonamide jaundice when the drugs are administered to patients with pre-existing jaundice not due to parenchymal liver disease.

3) The third variation of jaundice precipitated by the sulfonamides is represented by the cases with jaundice followed shortly by acute hemolytic anemia. So far only two cases have been reported. Our case was that of a 43 year old man admitted with severe erysipelas of the face who became jaundiced (icterus index, 33) in the first 24 hours after 6 grams of the drug. Because of the spreading erysipelas the drug was given for another 24 hours, totalling 12 grams. At this time the hemoglobin was 104 per cent. Jaundice was present for 48 hours and then a fulminating hemolytic crisis occurred. There was severe lumbar and

abdominal pain, vomiting and intense hemoglobinuria, bloody stools, and intensification of the jaundice (icterus index, 108). There was a concomitant drop of hemoglobin to 31 per cent with 1,800,000 red blood cells, normoblasts 8 per cent, and the cell volume became 16 per cent in one day. Recovery occurred after a stormy course and six transfusions totalling 3000 cc.

Spring and Bernstein (173) have described an almost identical case.

Greene and Hotz (164) suggest that the liver has a special affinity for sulfanilamide since in their fatal case they found the liver tissue contained 6 mg. per 100 grams as compared with only 1½ mg. in other organs. However, this type of distribution occurs in many other forms of poisoning which do not cause liver necrosis (115). The fact that poisoning may occur with all the drugs of the sulfonamide group indicates that it is the sulfonamide nucleus that is involved and not the side chain.

Liver Extract: We have recently seen twelve cases of jaundice in patients with pernicious anemia while under treatment with parenteral liver extract. The incidence in terms of percentage is extremely low. The clinical course in most of these patients was that of a mild simple jaundice lasting about six weeks. The liver function tests indicated some impairment.

In two very recent cases the course was of a subacute liver atrophy which lasted more than two months and was accompanied by distinctly abnormal liver function tests. They recovered ultimately. Parenteral liver therapy was resumed in all the patients after the jaundice had cleared up, without further untoward reaction (177).

It has been suggested that sensitization to liver extract occurs. However, the subsequent tolerance to the drug is not characteristic of allergy. It is worth bearing in mind that before the introduction of liver extract therapy, jaundice was well recognized as an occasional complication of pernicious anemia. It is possible that the undetermined factor which produced jaundice in those days may still be operating in the treated cases in spite of their good hematologic response and may be more frequent because more patients are being kept alive with liver extract.

THE NATURE OF DRUG HYPERSENSITIVITY

The toxic action of arsphenamines, cinchophens, sulfonamides, has been explained as a form of allergy. The basis for this claim is:

1) The relatively low incidence of jaundice considering the extensive use of these drugs.
2) The small dose precipitating jaundice in some cases after much larger amounts have been administered with apparent impunity.
3) The frequent latent period between the termination of therapy and the appearance of jaundi [illegible]
4) The occurrence of [illegible] especially [illegible] is urticarial.

Caution must be use[illegible] [illegible] as indicative of drug allergy for the following[illegible]

1) Occasionally, the [illegible] fc [illegible] dose (our case

sulfanilamide poisoning following the *single* deposition of the drug intraperitoneally).

2) Most chemicals which are consistently hepatotoxic have a long latent period (such as chloroform).
3) Previous liver injury of a subclinical type, such as occurs in chronic alcoholics or in serious debilitating illnesses, has been shown to predispose to jaundice from any of these drugs.
4) Most of the patients recovered from arsphenamine jaundice may continue their arsenotherapy although in a few instances this does cause recurrence of the jaundice. This tolerance is not characteristic of allergy.
5) Liver damage without jaundice is more frequent with the use of these drugs than is supposed. As a rule the existence of liver damage is not suspected before the signal of jaundice appears. Sudden death in the absence of jaundice has occurred not only in epidemics of simple jaundice and infectional acute yellow atrophy, but also during the administration of chloroform, cinchophen, arsphenamine, sulfonamide, and has been found to be explained by the extensive anatomic liver damage found at post-mortem examination. Cirrhosis of the liver following exposure to organic and inorganic arsenicals has occurred without jaundice.
6) Under some experimental conditions these drugs have been found to be hepatotoxic. Jaundice has been induced with arsphenamines in young dogs. Cinchophens have produced liver damage in cats; they have been shown to be "choleretic" in man, which may actually be an expression of mild toxicity. Thus far no experimental liver lesion has been induced with the sulfonamides. However, species differences must be considered.
7) The known behavior of the liver as a shock organ in experiments with anaphylaxis is an intense congestion and is different from the necrosis observed in clinical poisonings (174, 175).
8) Pharmacologic experiments have shown that arsenoxide, the product of the metabolized arsphenamines, is hepatotoxic. Also arsphenamine may be toxic because of its physicochemical properties as it agglutinates red blood cells and causes hemolysis in vitro (176).

In conclusion, hypersensitivity in a general sense exists, and some characteristics of the symptoms suggest allergy to the drugs. However, the strict criteria of allergic sensitization remain to be satisfied. It would be more correct to assume a non-allergic sensitivity, possibly on the basis of the previous state of nutrition, infection, or unknown metabolic abnormality.

In conclusion, we wish to bring together several points of interest which have developed in the course of our review.

1) Simple jaundice and acute atrophy of infectious origin are not unitary diseases, but may both be due to various viruses.

2) The occurrence of a latent period before the appearance of jaundice is common to many infections (a) and poisons (b):

(a) Epidemic acute yellow atrophy

Yellow fever
Leptospirosis
(b) Chloroform
Inorganic arsenicals
Acriflavine
Arsphenamines
Cinchophens
Sulfonamides
Acetylene tetrachloride
Dinitrophenol
Trinitrotoluene

There is some evidence that functional and anatomic liver damage is already going on during this clinically latent period.

3) A specific immunity follows several types of infectious jaundice: simple jaundice, yellow fever, spirochetal jaundice. In contrast is the greater susceptibility to many hepatotoxic agents after apparent recovery from jaundice due to a chemical.

4) Damage of the renal tubules is frequently associated with hepatic degeneration in:

Leptospirosis
Yellow fever
Transfusion of incompatible blood and other forms of acute hemolysis
Poisoning with acriflavine, carbon tetrachloride, phosphorus, mushrooms, arsphenamines.

5) Critical analysis of the claims for allergy as the mechanism for liver damage from certain chemicals (cinchophens, arsphenamines, sulfonamides) shows that most cases fail to fulfill the criteria for allergy. With our present knowledge it is wiser to consider these as non-specific hypersensitivity, possibly on the basis of an abnormal metabolism or of a nutritional defect.

6) Jaundice as a complication of burns has appeared only in the era of tannic acid therapy and may be related to it.

7) Attention is called to a possible latent jaundice-producing virus in human beings as indicated by the occurrence of jaundice some months after the injection of attenuated yellow fever virus mixed with pooled human serum and likewise after the injection of measles prophylactic pooled human serum.

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INTRAVENOUS ALIMENTATION WITH AMINO ACIDS

A Review

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Scientists have for years studied the problem of supplying a complete diet by vein A solution containing amino acids, vitamins, minerals and carbohydrate would constitute a preparation of value in postoperative therapy and in any condition in which the oral administration of food was impossible or was contraindicated The use of solutions containing dextrose, sodium chloride, salt combinations and vitamins which are given intravenously has become common practice, but the use of intravenous amino acids in the clinical practice of intravenous alimentation has occurred only within the last few years The major obstacle has been the preparation of amino acid solutions The use of pure amino acids was and is precluded by the cost Hydrolysates of various proteins, particularly casein, provide the only practical approach

I PROTEIN HYDROLYSATES AND THEIR PRODUCTION

Three general procedures are utilized acid hydrolysis, combined acid and alkaline hydrolysis and enzymatic hydrolysis Acid hydrolysis is the method of choice as it does not produce racemization of the amino acids This is important, as any preparation for amino acid therapy must contain the essential amino acids (lysine, tryptophane, histidine, phenylalanine, leucine, isoleucine, threonine, methionine, valine and arginine) and further must contain the natural optical isomer Of the ten indispensable amino acids, only l-tryptophane, l-histidine, l phenylalanine and l methionine can be replaced for growth purposes by their antipodes (1) Alkaline hydrolysis, using sodium hydroxide, potassium hydroxide or barium hydroxide, produces a rapid and complete reaction but racemization occurs, and this results in decreased utilizability of the hydrolysate Although racemization in strong acids can occur, the product of acid hydrolysis in the customary procedures is not racemized to any significant degree Many acids have been used as hydrolytic agents hydrochloric, sulfuric, hydrofluoric, hydrobromic, hydriodic, formic, phosphoric and acetic acids as well as acetic anhydride Hydrochloric acid and sulfuric acid are the preferred reagents and no advantages are to be found in the use of other acids The product of acid hydrolysis of protein consists of a mixture of alpha amino acids as well as ammonia and such non-protein prosthetic groups as were present in the original protein

Braconnot (2) in 1820 first prepared sulfuric acid hydrolysates of gelatin and meat His general procedure is now common practice Strong sulfuric acid (about 35%) is mixed with the protein and the mixture heated until hydrolysis is complete Barium hydroxide is added in amount equivalent to the sulfuric acid and the resultant barium sulfate filtered off The solution is then charcoaled to clear out the humin formed in the reaction Humin formation is one

INTRAVENOUS ALIMENTATION WITH AMINO ACIDS

A. Review

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Scientists have for years studied the problem of supplying a complete diet by vein. A solution containing amino acids, vitamins, minerals and carbohydrate would constitute a preparation of value in postoperative therapy and in any condition in which the oral administration of food was impossible or was contraindicated. The use of solutions containing dextrose, sodium chloride, salt combinations and vitamins which are given intravenously has become common practice, but the use of intravenous amino acids in the clinical practice of intravenous alimentation has occurred only within the last few years. The major obstacle has been the preparation of amino acid solutions. The use of pure amino acids was and is precluded by the cost. Hydrolysates of various proteins, particularly casein, provide the only practical approach.

I. PROTEIN HYDROLYSATES AND THEIR PRODUCTION

Three general procedures are utilized: acid hydrolysis, combined acid and alkaline hydrolysis and enzymatic hydrolysis. Acid hydrolysis is the method of choice as it does not produce racemization of the amino acids. This is important, as any preparation for amino acid therapy must contain the essential amino acids (lysine, tryptophane, histidine, phenylalanine, leucine, isoleucine, threonine, methionine, valine and arginine) and further must contain the natural optical isomer. Of the ten indispensable amino acids, only l-tryptophane, l-histidine, l-phenylalanine and l-methionine can be replaced for growth purposes by their antipodes (1). Alkaline hydrolysis, using sodium hydroxide, potassium hydroxide or barium hydroxide, produces a rapid and complete reaction but racemization occurs, and this results in decreased utilizability of the hydrolysate. Although racemization in strong acids can occur, the product of acid hydrolysis in the customary procedures is not racemized to any significant degree. Many acids have been used as hydrolytic agents: hydrochloric, sulfuric, hydrofluoric, hydrobromic, hydriodic, formic, phosphoric and acetic acids as well as acetic anhydride. Hydrochloric acid and sulfuric acid are the preferred reagents and no advantages are to be found in the use of other acids. The product of acid hydrolysis of protein consists of a mixture of alpha amino acids as well as ammonia and such non-protein prosthetic groups as were present in the original protein.

Braconnot (2) in 1820 first prepared sulfuric acid hydrolysates of gelatin and meat. His general procedure is now common practice. Strong sulfuric acid (about 35%) is mixed with the protein and the mixture heated until hydrolysis is complete. Barium hydroxide is added in amount equivalent to the sulfuric acid and the resultant barium sulfate filtered off. The solution is then charcoaled to clear out the humin formed in the reaction. Humin formation is one

of the major complications of acid hydrolysis. It is a black or brownish amorphous material which is thought to be formed from tryptophane when it condenses with an aldehyde (3). It will be apparent that the destruction of the essential amino acid, tryptophane, makes it imperative to reinforce any acid hydrolysate with this amino acid before use in intravenous alimentation. The value of hydrolysates which are not reinforced is very slight. Some tyrosine is also thought to be lost in production of this acid formed humin material, but it is only the tryptophane which is removed quantitatively. This loss of tryptophane through humin formation results regardless of the acid used in preparing the hydrolysate. Sulfuric acid, because of its easy removal as barium sulfate, offered certain advantages in the preparation of acid hydrolysates until the development by Johnson (4) of a process of removing the hydrochloric acid by adsorption on certain ampholyte resins. Since the development of this process, the use of hydrochloric acid provides the advantage as the danger of leaving traces of barium or excess sulfate in a sulfuric acid hydrolysate presents opportunities for complications. Further, sulfuric acid is non-volatile and therefore cannot be removed by vacuum distillation, whereas hydrochloric acid lends itself to this procedure. Silver salts were once used to remove the hydrochloric acid but this obviously is not practical for two reasons: traces of silver will remain in the solution, and silver salts are extremely expensive. White and Elman (5) have recently applied the principle of selective liberation of amino acids to the production of acid hydrolysates of protein suitable for intravenous administration. Calvery et al (6) had shown that tryptophane was liberated only in the final stages of hydrolysis, and it was apparent that the amino acid must be liberated before it could be destroyed. Calvery's observations formed the basis for the work of White and Elman, who find that after six hours heating only a portion of the tryptophane is liberated. This prevents the destruction of the greater part of the tryptophane and corrects one of the major difficulties of the use of acid hydrolysates. Schmidt (7) reviews the principles of selective liberation of amino acids and states that "tyrosine, cystine and arginine are among those amino acids which are readily liberated, while proline and phenylalanine are among the resistant ones." Oxalic acid has been used (8) and offers certain possibilities as a hydrolytic agent, since it can be removed as the calcium salt, and if calcium remains in the solution no harm is done; but the process involves the use of high pressure autoclaving, which is disadvantageous in large scale production.

The alkalies, sodium, potassium and barium hydroxide, produce rapid and complete hydrolysis but they also produce rapid and complete racemization. The use of these agents has one great advantage in that tryptophane is not destroyed, but the disadvantages are: racemization, destruction of cystine, and ammonia formation with resultant destruction of amino acids by deamination. Sahyun (9) has proposed the use of combination acid and alkaline hydrolysates in which the alkaline hydrolysates would supply the tryptophane, and the acid hydrolysates would supply the non-racemized amino acids.

The proteolytic enzymes provide the third group of agents used in the preparation of protein hydrolysates for intravenous alimentation. It was early recog-

nized that incomplete hydrolysis would result from the use of single enzyme preparations (10), and combinations were employed (10). This process represents a very mild treatment of the protein and amino acids and consequently results in very little destruction of the amino acids. There are several obvious disadvantages. The process is very slow, requires special equipment for large scale production, and it is impossible to remove the enzymes from the final hydrolysates. As proteins need not be broken down to the single amino acid stage to avoid immunological activity, it is apparent that enzymatic action, even if it carries the reaction only to the stage of polypeptides, results in a product which will be inactive immunologically. The Biuret test for dipeptide linkages should not be considered as an indication of the presence of protein split units capable of sensitizing the organism. The question remains as to the physiological or nutritive value of peptides as compared to individual amino acids. Further research will clear up this point. The clinician should insist on evidence of demonstrated immunological inactivity in the protein hydrolysate product he uses.

The combination of hydrolysates of various proteins offers great potentialities. It is well known that proteins differ in their amino acid composition, and this makes it possible to select for hydrolysis two proteins which would supplement each other's deficiencies. Thus, one would combine a low cystine, high tryptophane protein with a high cystine, low tryptophane protein and arrive at an ideal hydrolysate. At present, the difficulty of obtaining a variety of relatively pure proteins at low cost precludes the extensive utilization of this principle.

From the standpoint of the clinician, the ideal preparation must be one which is stable over prolonged periods in solution. This has been the chief difficulty in the commercial production of these items. Melanin formation, tyrosine precipitation and leucine-isoleucine precipitation: all complicated the problem. Titratable acidity is an important item, as amino acids are excellent buffers and therefore the pH of the final solutions must lie close to 7.0 in order to have a titratable acidity compatible with the necessity of using large volumes without danger of acidosis. The concentration of the amino acids must be such that the injection of 3 or 4 liters will supply more than the daily requirement. In this connection, the clinician must consider the need of supplying calorie value in some form; otherwise the amino acids will be burned for calories and not utilized for protein formation, etc. Thus far, no one has been able to stabilize amino acids and dextrose in the same solution, since the dextrose is aldehydic in nature and catalyzes humin formation. Simultaneous administration of glucose is the logical procedure. It has been suggested that added calorie value is not necessary, as the patient will burn his own fat rather than the amino acids; we believe that this is not accurate, but adequate evidence is not available.

II. ANIMAL EXPERIMENTATION INVOLVING THE PARENTERAL ADMINISTRATION OF AMINO ACIDS

In 1909, Abderhalden et al (11) reported that a positive nitrogen balance could be maintained by feeding digested lean beef per rectum. In the years 1912 to 1916, several interesting papers (12, 13, 14, 15) appeared on this subject. Note-

worthy among these was the work of Henriques and Anderson (13), who de onstrated that nitrogen equilibrium could be maintained with intravenou injected amino acids as the sole nitrogen intake. The nitrogen was supplied the form of a protein digest, hydrolized completely to amino acids, and inject in a slow stream into the neck vein of a goat. Thus, in the period 1909 to 19 it had been conclusively demonstrated that intravenous alimentation wi protein digests was feasible.

Most of the recent experimentation involving casein digests has centered the study of nitrogen retention and utilization in plasma protein regenerati and growth.

Cox and Mueller (16), using both enzymatic and acid protein hydrolysat demonstrated positive nitrogen balances in rats fed these preparations as t sole significant source of nitrogen in the diet. The acid hydrolysate was suppl mented by 0.2% of tryptophane. With both preparations, the nitrogen balan observed equalled that seen when casein was used as the nitrogen source. is interesting to note that the rats would not even eat the acid hydrolysate un it was supplemented with tryptophane and then they consumed it avidly. Wh the acid digest without added tryptophane was given by stomach tube, the a mals were found to fare better than if they received no supplements of ami acids. Another group of workers (17), using a sulfuric acid digest reinforc with tryptophane, were able to maintain normal growth for two weeks by eith intraperitoneal or intravenous administration.

Mixtures of amino acids, including histidine, isoleucine, leucine, lysine, pheny alanine, tryptophane and valine together with cystine or methionine or bot have been administered to dogs fed diets low in protein and it has been report that the nitrogen of these amino acids was retained completely or spared equiv lent amounts of tissue nitrogen (18). These investigators also demonstrate that a portion of the nitrogen of each of a number of amino acids, of creatine an of urea, administered to dogs partly deficient in protein has on occasion bee retained, but with most of the compounds the nitrogen was completely excrete Cystine, histidine and lysine seem to show selective retention of their nitroge It is to be emphasized that these single amino acids were catabolized and the nitrogen excreted as urea and ammonia. In some instances, an amino acid suc as glycine increased the nitrogen in the urine by an amount exceeding that whic the amino acid itself would supply, thus indicating that there had been an in creased catabolism of tissue protein. This would seem to be a point against th use of any incomplete amino acid preparation in any case in which positiv nitrogen balance and tissue or blood protein regeneration is the objective. I is not proposed to cover the literature on the effect of single amino acids o nitrogen balance but to point out the existence of evidence which indicates th probability that amino acids have interrelationships to one another which are a profound as those existing between vitamins and which indicate caution in th specific usage of single amino acids. Nielsen and Corley (19) demonstrate further that the white rat utilizes all the nitrogen of a mixture of amino acid consisting of threonine, histidine, isoleucine, leucine, lysine, methionine, phenyl

alanine, tryptophane and valine. These rats again were on protein low diets and not on protein deficient diets. Finally, Wolf and Corley (20) found that nitrogen balance could be established in rats on diets devoid of protein by the addition of an amino acid mixture consisting of histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane and valine. The omission of any one of these nine amino acids resulted in negative nitrogen balance, indicating the essentiality of each of the nine, both for growth as demonstrated by Rose (1) and for positive nitrogen balance as demonstrated by these workers. They emphasize the idea that one amino acid, if supplied in relatively great amounts, might replace one of the others or bring about a need for still another. Mueller et al. (21) using rats demonstrated the complete equality of enzymatic digests of casein and casein itself for growth and for the regeneration of serum protein. Elman (22) had injected a casein digest intravenously in dogs after massive hemorrhage and observed increase in serum albumin after 6 to 24 hours. Mueller et al. (21) suggest that the observations of Madden et al. (23), demonstrating protein synthesis following the use of each of 16 amino acids singly, may be due to the effect of chemical stimulation or mobilization of protein from body stores and not to a new synthesis from the ingested protein materials.

Burroughs et al (24) demonstrated that the adult rat may be maintained in nitrogen balance by supplying threonine, isoleucine, tryptophane, valine, methionine, tyrosine and norleucine. Thus it would seem that the amino acids necessary for growth are not the same as those required for nitrogen balance, which is not in accord with the report of Wolf and Corley (20). These investigators (24) suggest that the basis is the difference between rates of supply and demand with respect to the two functions. Investigations on the parenteral administration of amino acid hydrolysates began with the observation of Elman (22). The efficacy of both enzymatic digests and acid digests (supplemented by tryptophane and cysteine) in bringing about serum protein regeneration in bled dogs was demonstrated by Madden et al. (25). The digest was equally effective intravenously, subcutaneously or orally.

Sahyun (9) reported that greater utilization and deaminization followed the parenteral administration into fasting rabbits of an acid hydrolysate of casein (to which l-tryptophane and l-cystine had been added) than followed the administration of either glycine or partially racemized amino acids of an acid base hydrolysate of casein. This is in accord with expectation as the basic hydrolysate would contain racemized amino acids, only about one-half of which would be utilizable. This means that 75% of the amino acids in an acid base hydrolysate are utilizable and present in the natural form, whereas 25% are present in the non-natural isomer and therefore, excepting in the previously named instances, not utilizable. Sahyun (9) gives details of the methods of production of both acid and acid-base hydrolysates. Further confirmation is offered (26) of the availability of amino acids in enzymatic digests of casein for the regeneration of plasma protein. The digest was given intravenously to dogs on a nitrogen free diet when their protein stores were depleted and their plasma proteins lowered. These investigators (26) state that the "parenteral administered casein digest

constitutes an effective method of parenteral nutrition". Horvitz, Sachar a Elman recently reported (27) on the effectiveness of parenteral amino acids i the growth of rats. This same group (23) produced a chronic hypoproteinem in dogs by feeding a diet containing only sucrose and vitamin B concentrate Ringer's solution. The serum albumin levels dropped to 1.2 grams in thr weeks. They then administered 4 grams per kilogram body weight per day hydrolyized protein to these dogs for one week and found positive nitrog balances, serum protein regeneration and tissue protein regeneration. Th data indicate that 96.5% of the nitrogen retained by these animals was utiliz for the replenishment of the tissue proteins, while only about 3.5% could accounted for in the replenishment of serum albumin.

III. CLINICAL EXPERIENCE INVOLVING THE PARENTERAL ADMINISTRATION AMINO ACIDS

Studies of the therapeutic applicability of casein digests have proceeded alo lines similar to those pioneered in animal experimentation: nitrogen retenti and balance, serum protein regeneration, toxicity reactions, and use in indicat clinical entities associated with abnormalities in protein metabolism or sens tivity.

Abderhalden et al (11) and later Griesbach (29) used rectal alimentation wi hydrolyzed protein and found definite evidence of utilization. McClendon et a (30) reported positive nitrogen balances in patients given amino acids per rectur

Elman and Weiner (31) were the first to study the use of amino acids intravenous alimentation. They list as indications for amino acid therap any condition with hypoproteinemia, any condition in which oral alimentatic fails, e.g., when intestinal obstruction or repeated vomiting makes feeding in possible; any case with intestinal fistula or severe ulcerative colitis with diarrhe any condition in which it is desired to place the intestinal tract completely rest as a pre-requisite to healing following surgical procedures. These invest gators suggest nutritional edema as the most clearcut indication for parenter protein alimentation. In this connection, Meeray, Barden and Ravdin (32 have shown that with relief of hypoproteinemia both the edema and the obstru tive manifestations disappear in post-operative cases of pyloric obstructio Elman and Weiner (31) point out the impractical aspects of the use of plasma a a source of intravenous protein alimentation by showing that it takes two 50 cc. transfusions in a day in order to introduce 35 grams of serum protein, whic is a low protein intake. This amount of plasma would be difficult to obtain an expensive to use. They further point out that the protein replacement therap is for the replacement of proteins other than those of plasma, which means tha the transfused plasma protein must be broken down into amino acids and the resynthesized to tissue protein. By using protein digests, the amino acids ar supplied as such, since the initial hydrolysis is performed before administration. It is only necessary to point out a few of the functions of amino acids to emphasize the unlimited potentialities of this type of therapy. Glycine and cystine are used in the body to detoxify various toxic chemical agents. Tyrosine is the

probable precursor of adrenaline. Methionine is essential for the processes of transmethylation which are vital to body economy. Amino acids and protein exert a vitamin sparing action. Amino acids are not only required as the building stones of tissue protein but go to form many of the hormones of the body and sometimes function as tripeptides (glutathione) in enzyme systems. This is only a partial list and serves to indicate some points at which deficiency of amino acids would disturb physiological function. Elman and Weiner (31) after preliminary observations injected from 0.5 to 2.0 grams of amino acids per kilogram of body weight. They used an enzymatic digest reinforced with 2% each of cystine and tryptophane. The digest was made up in 10% solution and then diluted with either 5% or 10% dextrose, so that the final solution contained 20 grams of amino acids and 80 grams of dextrose per liter. Given over a period of two hours the 20 grams of amino acids did not spill over into the urine and produced no objective reaction. They report that some patients noted peripheral vasodilatation as evidenced in a sense of warmth. No parenchymatous changes in the liver or kidney were noted in any of their patients that came to autopsy. One of these had received two grams of amino acids per kilogram for a period of ten consecutive days. They state that their most dramatic effects came in the relief of nutritional edema. For a 70 kg. adult 1,600 calories as glucose in the absence of fever (more if fever is present) is recommended as an adjuvant. This indicates that 400 grams of dextrose and amino acids could easily be given. 0.5 gram of amino acids per kilogram is suggested as adequate, although they emphasize the necessity of greatly increasing this level in many conditions. Shohl et al. (33) used casein digests to maintain positive nitrogen balance in their clinical practices; however, they report that temperature elevations were always noted. Somewhat similar results were reported by Fetzer (34) using an enzymatic digest of casein. Further confirmation of the work of Elman and Weiner (31) was reported by Altshuler, Hensel and Sahyun (35), who used both subcutaneously and intravenously a casein digest reinforced by cystine and tryptophane in normal and postoperative patients. They observed no untoward reactions and found that the amino acid mixture was almost completely utilized. The solution these investigators used contained 1.0% nitrogen, equal to 7 grams of amino acids per 100 cc., 5% glucose and 0.7% saline. It had been reinforced with 1.5% tryptophane and 1.5% cystine. It was finally diluted with an equal amount of sterile water. 1000 cc. of this fluid was injected in 4 or 5 hours. Farr and MacFadyen (36) reported the complete utilization of amino acid hydrolysates in nephrotic children. They used hydrolysates produced enzymatically. Extending his previous report on the use of enzymatic casein digests Farr (37) used a 10% solution of the preparation and found that his patients showed flushing and had sensations of warmth on administration. If he gave 5 grams in a period of 30 minutes he got no nausea; however, if 10 grams were given in 20 minutes he noted the common occurrence of nausea. It is his conviction that intravenous amino acids provide better nitrogen assimilation than the feeding of protein—this in nephrotic children. He could detect no increased effect of amino acids when given simultaneously with glucose. In a later paper Farr et al (38)

further extended the study of the use of intravenous amino acids in children with nephrotic syndrome. The casein digest and glucose were given in 10% solution, the total dose divided into three equal daily doses. In the course of their investigations they administered 210 to 294 grams of the hydrolysate during a single week with no demonstrable permanent effect on the plasma amino acids. Flushing of skin, sensation of warmth, cramps, headache, vomiting were seen in some instances following the use of amino acids intravenously. These could be avoided by reducing the rate of injection. In these patients, the toxic nitrogen metabolism of Farr did not occur. There was an absence of negative nitrogen balances preceding a crisis and the assimilation of increased quantities of nitrogen would appear to have resulted both from a decrease in the toxic nitrogen metabolism and the administration of intravenous amino acids. Plasma protein was not increased in these patients by the administration of amino acids. One effect of intravenous amino acids in nephrotic patients was to increase the urea clearance when renal function was normal. Farr suggests that the development of a positive blood stream infection in nephrotic patients is a definite indication for intravenous amino acid therapy. He further suggests (39) the use of amino acids in other conditions of hypoamino acidemia aggravated by some acute disturbance. He reports in this communication results on 17 nephrotic crises complicated with pneumococcal bacteremia with not a single death, whereas before the use of amino acid the mortality in such crises was 60%. Shohl and Blackfan (40) have conducted the only study thus far reported on the use of crystalline amino acids in intravenous alimentation in humans. No advantage was claimed for mixtures of crystalline amino acids over casein hydrolysates from the standpoint of nitrogen balance. The rise in temperature which accompanies the intravenous administration of amino acids was found to be caused to the same degree by the use of mixtures of crystalline amino acids and casein hydrolysates. They gave from 4 to 6 grams of amino acids per kilogram per day as they felt these amounts were necessary for adequate nitrogenous nutrition. They used an enzymatic casein hydrolysate containing 11.9% nitrogen of which over 60% was present as amino nitrogen. The mixture of crystalline amino acids used by these workers consisted of glycine, 3 grams; dl-alanine, 3.8 grams; dl-valine, 16.0 grams; l-leucine, 9.0 grams; dl-isoleucine, 8.0 grams; dl-norleucine, 2.5 grams; l-proline, 8.0 grams; l-hydroxyproline, 2.0 grams; dl-phenylalanine, 7.8 grams; d-glutamic acid, 22.0 grams; d-aspartic acid, 4.1 grams; dl-serine, 3.0 grams; l-tyrosine, 2.0 grams; l-cystine, 0.5 grams; l-histidine, 3.4 grams; d-arginine hydrochloride, 6.35 grams; d-lysine dihydrochloride, 11.55 grams; l-tryptophane, 2.25 grams; dl-methionine, 3.5 grams; d-threonine, 2.5 grams; added sodium bicarbonate, 12.86 grams; making a total of 134.31 grams. When only the dl- form of an amino acid was available, they added twice the basic amount in order to provide adequate active isomer. The final solution contained 5% amino acids, 5% glucose and 0.45% saline. It was administered intravenously into a scalp vein at a rate not exceeding 200 cc. per hour. When amounts greater than 15 grams were given to small infants, a febrile reaction usually occurred. In one infant weighing 4.5 kilos, the injection

of 840 cc. of the solution containing 22.0 grams of amino acids provided positive nitrogen balance. The above data are listed in detail as they provide a means of calculating a dosage of single amino acids which will provide adequate nitrogen balance. This does not mean that these values are minimal nor does it mean that they are optimal. For example, tryptophane must be added to acid hydrolysates and some value must be assigned to a minimum acceptable level from present data. Calculations show that the hydrolysate must supply per kilogram per day not less than 82 milligrams; however, as casein contains 2.2 per cent of tryptophane, this would mean that 3.7 grams of casein per kilogram, or for a 70 kilogram patient a daily intake of some 259 grams would be necessary. As this is approximately four times the accepted daily intake of 70 grams of protein for an adult human being, it is probable that the tryptophane requirement is approximately 1.0 gram per day per 70 kilos. This calculation has been made in order to emphasize the point that the amounts of individual amino acids used in the work of Shohl and Blackfan are not minimum doses for optimal effects but, rather, working guesses which served the purpose of the experiment.

The indications are for extended investigation to determine the minimum amounts of the essential amino acids necessary for positive nitrogen balance, serum protein regeneration, etc., and to determine how much of the daily nitrogen requirement can be supplied by non-essential amino acids such as glycine or by other nitrogenous compounds (urea, etc.). This is the line of investigation that may permit the use of mixtures of crystalline amino acids, a practical and inexpensive method. Elman (41) reported on the extension of his original investigations in a series of 35 adults, who were injected intravenously with a solution containing glucose, amino acids and electrolyte as the sole source of alimentation. The maximum amount of nitrogen administered was 9.6 grams per day, the calories, 1,600. Evidence of utilization was shown by increases in serum protein concentration, achievement of nitrogen balance, and clinical improvement, particularly after serious operation. He considers the large increase in nitrogen in the urine of patients postoperatively as being a manifestation of the toxic destruction of protein which is prevented or replaced by the intravenous administration of amino acids. Elman (41) used a solution containing 2.0% amino acids and 8% glucose. In eight hours of continuous venoclysis he administered 4000 cc. which contained 80 grams of amino acids and 1,600 calories, plus 10 grams of sodium chloride. The nitrogen concentration of the final solution would be 0.24 grams per cent. He states that there is but a slight tendency toward thrombosis when 2% solutions are used and that he has injected 2,250 cc. of a 10% glucose solution containing 2% amino acids in five hours without reaction. He mentions another product which contained 2.5% hydrolyzed casein and which permitted him to give 1000 cc. in one hour with no reaction. The large output of nitrogen, as high as 26 grams per day, seen in postoperative patients is, according to Elman, an indication that that much nitrogen is required in the alimentation procedure but that any amount exerts a beneficial effect in these patients. He stresses the necessity of adequate calorie intake in order to obtain the maximum effectiveness of the amino acids. Messinger (42) has

further extended the study of the use of intravenous amino acids in children with nephrotic syndrome. The casein digest and glucose were given in 10% solution, the total dose divided into three equal daily doses. In the course of their investigations they administered 210 to 294 grams of the hydrolysate during a single week with no demonstrable permanent effect on the plasma amino acids. Flushing of skin, sensation of warmth, cramps, headache, vomiting were seen in some instances following the use of amino acids intravenously. These could be avoided by reducing the rate of injection. In these patients, the toxic nitrogen metabolism of Farr did not occur. There was an absence of negative nitrogen balances preceding a crisis and the assimilation of increased quantities of nitrogen would appear to have resulted both from a decrease in the toxic nitrogen metabolism and the administration of intravenous amino acids. Plasma protein was not increased in these patients by the administration of amino acids. One effect of intravenous amino acids in nephrotic patients was to increase the urea clearance when renal function was normal. Farr suggests that the development of a positive blood stream infection in nephrotic patients is a definite indication for intravenous amino acid therapy. He further suggests (39) the use of amino acids in other conditions of hypoamino acidemia aggravated by some acute disturbance. He reports in this communication results on 17 nephrotic crises complicated with pneumococcal bacteremia with not a single death, whereas before the use of amino acid the mortality in such crises was 60%. Shohl and Blackfan (40) have conducted the only study thus far reported on the use of crystalline amino acids in intravenous alimentation in humans. No advantage was claimed for mixtures of crystalline amino acids over casein hydrolysates from the standpoint of nitrogen balance. The rise in temperature which accompanies the intravenous administration of amino acids was found to be caused to the same degree by the use of mixtures of crystalline amino acids and casein hydrolysates. They gave from 4 to 6 grams of amino acids per kilogram per day as they felt these amounts were necessary for adequate nitrogenous nutrition. They used an enzymatic casein hydrolysate containing 11.9% nitrogen of which over 60% was present as amino nitrogen. The mixture of crystalline amino acids used by these workers consisted of glycine, 3 grams; dl-alanine, 3.8 grams; dl-valine, 16.0 grams; l-leucine, 9.0 grams; dl-isoleucine, 8.0 grams; dl-norleucine, 2.5 grams; l-proline, 8.0 grams; l-hydroxyproline, 2.0 grams; dl-phenylalanine, 7.8 grams; d-glutamic acid, 22.0 grams; d-aspartic acid, 4.1 grams; dl-serine, 3.0 grams; l-tyrosine, 2.0 grams; l-cystine, 0.5 grams; l-histidine, 3.4 grams; d-arginine hydrochloride, 6.35 grams; d-lysine dihydrochloride, 11.55 grams; l-tryptophane, 2.25 grams; dl-methionine, 3.5 grams; d-threonine, 2.5 grams; added sodium biearbonate, 12.86 grams; making a total of 134.31 grams. When only the dl- form of an amino acid was available, they added twice the basic amount in order to provide adequate active isomer. The final solution contained 5% amino acids, 5% glucose and 0.45% saline. It was administered intravenously into a scalp vein at a rate not exceeding 200 cc. per hour. When amounts greater than 15 grams were given to small infants, a febrile reaction usually occurred. In one infant weighing 4.5 kilos, the injection

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also reported on the use of amino acids in the nephrotic stage of nephritis to promote the regeneration of serum proteins. In two cases, he noted a marked rise in the level of serum protein, mainly in the albumin fraction; in one case, no response was elicited. It is of interest to mention the work of Hill (43) with allergic infants who were fed casein hydrolysate with excellent results. There would seem to be potentialities for intravenous alimentation with amino acids in many of these cases. Studies on the utilization of an enzymatic digest of casein were carried out by Hartmann et al. (44), who used these digests both orally and intravenously with success. Orally, the digest offered an excellent substitute for meat, milk, eggs, etc. They report some severe reactions, such as vomiting, chills and fever, or phlebitis with edema but mention that the use of another amino acid preparation, recently supplied to them, resulted in the disappearance of these reactions and the occurrence of only mild reactions in a few instances. They did not obtain positive nitrogen balances in their studies, in which intravenous alimentation provided the sole source of nitrogen as intercurrent infections occurred which prevented continuation. It seems improbable that their difficulties could be attributed to other than improper preparation of the casein digests. The most recent paper of Elman and Weiner (45) includes studies conducted on 312 patients using some 1113 liters of glucose solution containing a mixture of amino acids. The injections were well tolerated and gave evidence of clinical benefit. They state that from 8 to 12 grams of amino acid hydrolysate can be injected per hour intravenously with complete safety. It is further suggested that it is possible to inject 40 grams per hour into a 70 kilo patient with no untoward reactions. This applies to the use of properly prepared enzymatic hydrolysates containing from 2.5 to 5.0% amino acids at a pH of 4.5. Brunschwig, Clark and Corbin (46) emphasize the value of this procedure in the surgical patient. They used an enzymatic preparation at 10%, diluted with equal parts of 5 or 10% glucose, or diluted with two parts of isotonic saline. They reemphasize the necessity of supplying caloric value in order to prevent the catabolizing of the amino acid as sources of energy. This is, of course, easily done with simultaneous administration of glucose. 500 cc. of 10% amino acid solution was given in from 30 to 45 minutes to some patients with complete absence of reactions. Icteric patients would seem to be unable to tolerate intravenous amino acids as well as other types of patients. Loss of from 3.81 to 175.79 grams of nitrogen in ten day periods following major surgical procedures was recorded. They state that the most important factor in the nitrogen loss is the restricted ingestion of food combined with the general physiological disturbances accompanying a major surgical procedure. Intravenous alimentation with casein digests and glucose reduced or even prevented postoperative net loss of nitrogen, sparing the organism the effects of excessive protein metabolism. They recommend intravenous administration of casein digests as a means of forced nitrogenous nutrition whether surgical procedures are contemplated or performed. These investigators have now (47) reported on the use of preparations for parenteral use containing dextrose, casein digest and emulsified fat. They are the first to use a

combination of the three primary types of foodstuff in intravenous alimentation. For a period of 17 days they maintained a patient with a cervical esophagus using intravenous alimentation for all nutrient excepting vitamins and liver extract which were given into a sinus in the upper end of the esophagus. The patient showed a positive nitrogen balance and maintained his weight.

Although interesting from an academic standpoint, it is doubtful if homogenized fat emulsions have any advantages over glucose solutions as a source of calories. It is true that per gram the fat provides much more caloric value, but it is also true that the caloric requirement can easily be met by the use of glucose and there is no comparison as to ease of preparation, expense of preparation, purity of material and safety in usage.

Thus, in summary, it can be stated that intravenous alimentation with amino acids is a perfectly safe, sound clinical procedure. The predominating opinion would seem to favor solutions containing from 2 to 5% of amino acids, but 10% solutions have been safely used. The rate of infusion is important; it is certain that 20 grams of amino acid can be given in two hours with no spilling over into the urine. Flushing, feeling of warmth and other mild symptoms are sometimes seen following or during the administration of protein hydrolysates; these can be prevented by decreasing the rate of infusion of the hydrolysate. The only condition contraindicating the use of amino acids thus far reported is icterus. The indications for intravenous alimentation with amino acid are conditions with hypoproteinemia, conditions in which oral alimentation is impossible, and for pre- and post-operative cases. This in no way implies that the list of indications is complete; it is logical that extensions be made in this line of investigation now that the procedure has been established to be safe and now that the protein hydrolysates are available. The daily dose to be used varies from 35 to 130 grams per 70 kilo patients, with an average around 70 grams of amino acids. The best means of determining the requirement is to measure the 24 hour excretion of nitrogen and supply that amount plus 10%. Adequate caloric intake must be provided in order that the above values are valid. Glucose given in 10% solution to provide 1600 calories will permit the body to utilize the amino acids for protein synthesis rather than for the production of energy. It is to be emphasized that the amino acid hydrolysate must contain all of the essential amino acids. Enzymatic hydrolysates, reinforced acid hydrolysates and alkaline acid hydrolysates all afford certain advantages, and it is probable that each type of hydrolysate can be used with equal effectiveness. Post-mortem examinations have disclosed no histopathology attributable to the use of amino acid preparations in intravenous alimentation.

IV. RECENTLY REPORTED ITEMS OF INTEREST IN THIS PROBLEM

It is highly probable that those conditions in which hypoamino acidemia exists are indications for protein digest therapy. Farr, McCarthy and Francis (48) have demonstrated lowered plasma amino acids in pneumococcus pneumonia. They did not find any abnormality in amino acid plasma concentration in scarlet fever or measles. No attempt has been made to cover the literature

on this subject, as we wish only to indicate an avenue for the extension of the problem.

Holt and his coworkers (49, 50) have recently reported on the effect of single amino acid deficiencies in human beings. These workers established tryptophane as a dietary essential for human beings without which nitrogen equilibrium cannot be attained. Lysine also was demonstrated to be essential for the maintenance of nitrogen equilibrium. In one female, the withdrawal of lysine caused a practically complete cessation of menstruation. In three male subjects kept on an arginine free diet for nine days, the sperm count was reduced to one-tenth of normal. It is suggested by these investigators that a temporary deficiency of arginine in the diet is met by atrophy of spermatogenic tissues because of the high content of arginine in these tissues. This presents the interesting possibility that the deficiency of a given essential amino acid will result primarily in the destruction of the tissue richest in that amino acid.

For purposes of serum protein regeneration, positive nitrogen balance and general intravenous alimentation, it is apparent that individual amino acids will not serve the purpose; however, it is not desired to imply that therapy with specific amino acids is an illogical or improbable procedure. On the contrary, it is evident to anyone associated with medical biochemistry that the existence of specific single amino acid deficiencies is to be expected and that these deficiencies will respond to therapy with the single amino acid. As with vitamins, these single amino acid deficiency states will probably be complicated by coexisting deficiencies of other amino acids. The analogy between vitamin and amino acids leads to these prognostications. The value of therapy with single amino acids has been demonstrated by Rabinowitz (52), who demonstrated the effectiveness of methionine in controlling spontaneous bleeding in the acute form of essential thrombocytopenic purpura.

In conclusion, a quotation from an editorial (51) in the Journal of the American Medical Association in 1941 is in order. It states, "for the problem of meeting the protein requirements of the entire organism is essentially one of supplying an adequate mixture of amino acids in sufficient quantity". The editorial continues, "The problem of a convenient and economical method of preparing a protein hydrolysate suitable for injection does not, however, appear to be insurmountable."

The field of intravenous alimentation with amino acid preparations has hardly been touched; it offers tremendous potentialities for clinical research, and its present clinical uses have demonstrated its value and safety.

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IMMUNITY IN MALARIA*

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INTRODUCTION

It has been witnessed many times that the rapid accumulation of essential knowledge concerning any disease has all too frequently been followed by a stagnant period where further explorations seem unnecessary. An outstanding example of this is found in malariology—the disease was recognized as a clinical entity in the time of Hippocrates who differentiated the intermittent types of fever. With the exception of the discovery of quinine in the 17th century little progress was made until 1884 when the causative agent was discovered by Laveran, the Frenchman. Then suddenly, at least when compared to past developments, the intricate details of the life cycle of the parasite in man and the anopheles mosquito and the mode of transmission were understood through the brilliant studies of Manson, Ross, Grassi, MacCallum and others. So that by 1900 it seemed quite safe to prophesy the early control or perhaps eradication of this disease which was retarding the progress of man in a major part of the world. There were so many vulnerable points to attack. Forty years of effort primarily devoted to application of various modes of control brings us up to date with the realization that in order even to keep pace with this disease, more information and new approaches must be had because malaria as a world problem is not diminishing. As Hackett (1) said in 1937, "the simplicity of the theory of malaria control is surpassed only by the difficulties involved in its application."

Some of the perplexities of the malaria problem are concerned with those factors associated with the acquisition and development of immunity, a phase of malariology sadly neglected. Because of a tendency to use multiple and new terminology in order to explain the different immunological manifestations of a malarial infection, there has been created the impression in many minds that the development of immunity does not follow the pattern observed with most other infectious diseases but in many respects possesses an individual behavior of its own. However, as more and more factual information accumulates, one is impressed with the similarity between the immune responses of the host to the malarial plasmodium and to those observed with other pathogenic agents—be it virus, bacterium, rickettsia, or spirochaete.

It has been generally accepted that a malarial infection confers a degree of immunity upon its host after the acute attack has subsided. Also it is well-known that the chronic infection or latent period once established is represented by an equilibrium between the parasite and the host which, evidenced by frequent relapses, at first fluctuates widely, becomes stabilized and ultimately disappears. The mechanism responsible for the conversion of an acute attack

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into a chronic one, the character and location of the various immune responses, and the duration of infection and immunity are all questions of considerable controversy.

The study of the immune responses in human malaria has been retarded because of two serious handicaps; first the inability to infect lower animals with any of the human plasmodia and secondly the lack of a method for the cultivation of the parasite. Another less serious difficulty, whether in man or experimental animal, has been the varied responses of the host to the parasite. Fortunately the behavior of certain experimental infections, especially some of the monkey malarias, so closely simulates the disease observed in man, that valuable inferential evidence can be obtained.

The nature of malarial immunity since the time of Golgi (2) was considered to be chiefly cellular in nature largely because of the intense activity of certain fixed tissue elements during acute or chronic infections. More recently Taliaferro and his associates (3, 4, 5) have clearly elucidated the cellular immune process when they showed that the macrophages of the spleen, liver and bone marrow destroyed the malarial parasites after they have been concentrated in these organs. The reader is referred to the original publications for the important details of this careful study.

Although recognizing that the chief concern of the cellular elements of the body is to collect and remove the organsims from the blood stream, there are numerous characteristics of a malarial infection which can best be explained on the basis of coöperating antibodies. In avian malaria, for example, when recovery occurs spontaneously, it develops with such rapidity that it is commonly referred to as a crisis. Since active phagocytosis commences at the onset of infection, it seems evident that an additional factor is playing a part in the defense of the host. There are many features of the different malarial infections indicative of a humoral immunity but studies directed toward its demonstration and role were either inconclusive or entirely negative. A review of the earlier reports emphasizes the lack of suitable hosts or infections for such studies (6, 7, 8, 9, 10). Fortunately in 1927 a malarial parasite, since named *Plasmodium knowlesi*, was isolated from a cynomologos monkey in Java (11). This aroused no special interest until later when it was noted that if it was inoculated into the rhesus monkey, the animal invariably became infected and the resultant infection practically always terminated fatally.

However, if antimalarial drugs were administered early in the course of the disease, the infection became chronic and remained so for many months, or even years, without further therapy. Daily examination of blood smears following treatment revealed parasites for two or three months, after which time they would usually disappear and only reappear for brief intervals. Superinfection by injecting large numbers of parasites intravenously was without visible effect; they were removed in a few hours from the peripheral blood stream. It is easily seen that *Plasmodium knowlesi*, either with its lethal effect in the rhesus monkey or with its chronic character following treatment, afforded an unusual opportunity to attempt the demonstration and study of humoral immune substances

in the blood serum, especially since this infection also presented much indirect evidence to support the belief that a humoral factor in malarial immunity existed. For example, a rhesus monkey develops such an effective immunity following recovery from a treated acute attack of *Plasmodium knowlesi* malaria that it can completely remove many billion viable parasites within a few hours after they have been injected into the blood stream. Ordinarily one, or at least less than ten, parasites constitute the minimal infective dose. Yet when this highly immune animal is reinoculated with a few morphologically similar but less virulent *Plasmodium inui* parasites, an infection ensues which is typical in all respects of that observed in a normal monkey of the same species. Likewise a monkey with a chronic *Plasmodium inui* infection and a high degree of immunity to the homologous parasite will develop a characteristic fatal *Plasmodium knowlesi* infection subsequent to inoculation with the latter parasite. The finding that recovery from an attack of malaria leaves the host with a solid immunity against the parasite that produced the infection yet practically as susceptible as a normal to a closely related strain would indicate that something in addition to a highly activated macrophage system was essential to malarial immunity. It was shown with Kumm (12), in 1937, for the first time that specific protective antibodies could be demonstrated regularly in the serum of rhesus monkeys with chronic *Plasmodium knowlesi* infection. This was evident in protection tests which showed marked prolongation of the usually fatal infections or transference into a chronic form without the aid of chemotherapy.

This demonstration of the passive transfer of immunity has since been confirmed by Manwell and Goldstein, Hegner and Eskridge, and Taliaferro and Taliaferro with avian malaria (13, 14, 15, 16), and Mosna, and Mulligan et al. with monkey malaria (17, 18). Similar tests involving the use of human immune malarial serum could not be attempted because of the lack of any experimental animal susceptible to the human parasite. This handicap was overcome in an indirect manner by utilizing the discovery of Knowles and Das Gupta (19) who succeeded in producing active malarial infections of many days' duration in three human volunteers following the inoculation with *Plasmodium knowlesi*, which, incidentally, made it the only known malarial parasite infectious for both man and lower animal. By inoculating patients suffering from general paresis with this organism for its therapeutic effect, it was possible to take serum samples from the patients at various intervals during their malarial infection and use them in protection experiments with the common rhesus monkey as the test animal. The results show that man like the experimental animal acquires protective substances in his serum during the course of a knowlesi malarial infection (Coggeshall, 20). That these substances are acquired as the result of the infection is shown by the fact that they are not present until the chronic stage of the infection has been reached.

The protection test with *Plasmodium knowlesi* is very unwieldy, requiring large numbers of rhesus monkeys, and for this reason it is only practical to determine relatively gross differences in the potency of immune serums. Since the results of the protection tests are not as clear-cut as those commonly used

in the study of viruses an effort was made to determine quantitatively the relationship between the amount of immune serum and number of viable parasites in the inoculum (Coggeshall and Eaton, 21). By titration it was found that a very definite relationship existed and it was possible to obscure completely the protective effect of a potent immune serum unless the number of parasites was kept at a minimum. This finding probably explains the failures in previous attempts to demonstrate passive immunity. The difficulty in detecting protective antibodies has led to the assumption that they are present in extremely low dilutions, which may not be the case. Presumably the malarial parasite has a protective covering in the red cell membrane which prevents an optimal union between the organism and the antibody. Consequently the antibodies may be present in appreciable concentrations, yet unable to exert their maximal influence against the parasite.

There can be no doubt of the specificity of the malarial protective antibody because protection tests with *Plasmodium knowlesi* immune serum of known high potency are exceedingly effective against the homologous parasite yet fail to exert any influence when used in the same test against the milder heterologous organism, *Plasmodium inui*. This lack of cross immunity to species is not surprising in view of certain findings of Mulligan and Sinton who have shown that it is possible immunologically to differentiate strains of *Plasmodium knowlesi* (22). There are five of these so-called strains that have been isolated from naturally infected cynomolgos monkeys captured in the same locality and there is no reason to suspect that these are the only ones. They are morphologically indistinguishable and all produce fatal infections unless treated. However, if any one of them is used to infect a monkey, a temporary protection against any of the other four strains is noted immediately following the recovery from the acute attack, but six months later the animal is immune only to the particular strain used in the primary infection. When reinoculated with any other of the strains it will not survive unless treated. This lack of a permanent cross immunity even with strains so closely related that they hardly deserve such recognition clearly indicates that immune serum resulting from these respective strains would be no less specific in a protection test; however the actual demonstration of strain specificity with serum has yet to be accomplished. Also the lack of cross immunity in monkey malaria of strains within a species has its counterpart in human malaria. In the important studies of James (23), and of Boyd, Carr and Rozeboom (24), it was noted that recovered individuals were highly immune to the strain used to initiate their therapeutic malaria but they were as susceptible as any normal when exposed to the bite of a mosquito infected with the same species of parasite originating from a different locality. The strain immunity in these instances indicated that individuals were immune only to those strains existing within relatively limited areas.

Suffice it to state at the moment that the accumulated data furnish definite proof of the existence of the humoral factor in malarial immunity. Its importance in the defense mechanism has yet to be evaluated but the fact that man or animal only retains an immunity to the strains with which they have had actual

experience must mean that some factor or factors in addition to a highly efficient cellular defense mechanism is responsible for the marked degree of specificity encountered with malarial infections.

The concentration of protective antibodies in a chronic malarial infection is constantly shifting as it responds to the rate of multiplication of the parasite. For example, the rhesus monkey will ordinarily harbor a chronic *Plasmodium knowlesi* infection for a year or more during which time it undergoes numerous parasitic relapses and spontaneous recoveries. If serum is obtained for protection tests from animals immediately preceding one of these relapses and again

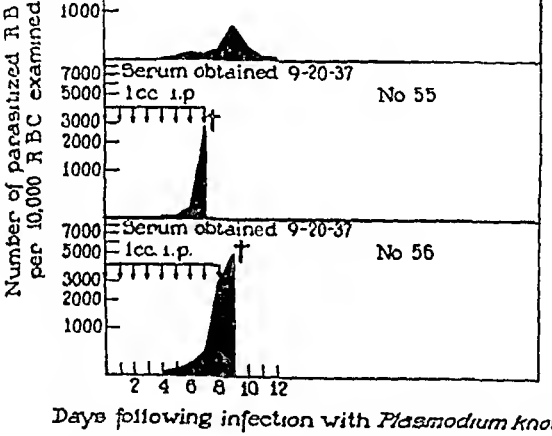

FIG. 1. SERUM IMMEDIATELY FOLLOWING RELAPSE SHOWS STRONG PROTECTIVE ACTION WHILE SERUM PRECEDING RELAPSE IS LACKING EFFECT

when the parasites have disappeared, there is usually a lack of demonstrable activity in the specimens preceding the relapse and a marked protective effect in the post-relapse serum sample. An example of the loss of humoral antibodies before a relapse is shown in figures 1 and 2. This phenomenon was first noted when obtaining an artificial elevation of the titre of protective antibodies in animals with chronic infections following superinfection with several billions of parasites at approximately weekly intervals over a period of three months (Coggeshall and Kumm, 25). As expected, in view of similar stimulating procedures with other infectious agents it is possible to produce a hyperimmune serum of high potency when compared to pools of serum obtained from similar animals that have not been superinfected. However, it was unexpected to find

that the post-relapse serum from monkeys in early convalescence frequently was even more potent than the serum from animals that had been successfully hyperimmunized. But when considered from the standpoint of the amount of available antigen to stimulate antibody production it is not so surprising because even with a very moderate relapse showing less than 0.5 per cent of the red cells parasitized there are no less than 500 billion parasites released during each 24-hour sporulation period. A parasitic relapse persists for a minimum of three days and usually longer. Thus the amount of antigen in the form of viable parasites present in these spontaneous relapses is many hundred fold in excess

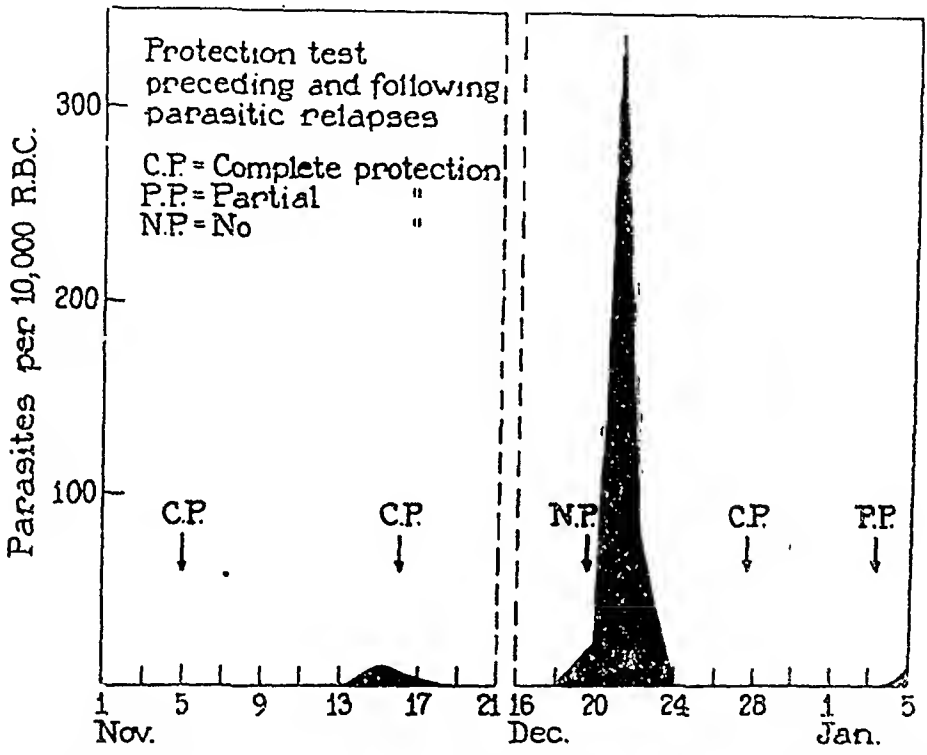

FIG. 2. SHOWING RELATIONSHIP BETWEEN POTENCY OF IMMUNE SERUM AND OCCURRENCE OF SUCCESSIVE RELAPSES

of that practicable or even possible to introduce artificially. This finding in no way discloses the factors which permit the relapse to occur but indicates that the relapse is beneficial when considered as a mechanism which serves to bolster the immunity of a host. In other words it is a process of auto-hyperimmunization rather than a phenomenon wholly detrimental to the host.

The evidence cited above of the ability to produce a hyperimmune serum with repeated inoculations of massive numbers of living parasites is an indication that the host is responding although there is no change in the numbers of circulating parasites. Because if one measures the response of the spleen with the aid of an oncometer following massive superinfection there is a rapid increase of about twice the original volume with almost as rapid return to the starting

level (Coggeshall, 26, and Afridi, 27). In nature the same result probably is obtained through the constant exposure of individuals to the bites of infected mosquitoes which may serve as a stimulus to maintain a high level of immunity. It is very commonplace in heavily infected areas to find fairly intense blood stream infections in individuals in the absence of, or with only a minimum of clinical symptoms. In spite of an infection of sufficient intensity to produce severe reactions in the less exposed, these individuals are not obviously ill. The presence of circulating parasites in the blood of the individual does not indicate a low-grade immunity or a breakdown in the defense mechanism. This thesis has experimental support in the laboratory where a marked protective effect can be obtained with serum taken from a highly immune monkey showing circulating parasites—actually it is possible to use the same monkey as the source of both the immune serum and the parasites for a positive protection test.

Although the exact rôle of the protective antibody has not been demonstrated the most likely possibility is that it acts as an opsonin and alters or sensitizes the parasite so that it becomes more susceptible to phagocytosis.

There is considerable experimental evidence to uphold the assumption that an immune serum can act upon a parasite without the participation of the cells of the host. A union between the parasite and the antibody undoubtedly occurs *in vitro* because a greater protective effect is obtained when the mixture is incubated at 37°C. for one-half hour as compared to the protection obtained when the two components are mixed and injected without incubation (Coggeshall, 28 and Taliaferro, 29). Actually some sera from highly immune animals will completely inhibit the infectiousness of the parasite when the incubated mixture is inoculated into a susceptible animal. Examination of these parasites reveals no morphological changes although this does not mean that it is possible visually to distinguish living from killed organisms and is not evidence that a direct killing effect has resulted. Also it can be shown that an antigen-antibody reaction takes place *in vitro* by absorbing the protective antibodies from an immune serum by the addition of living parasites, and then removing both by centrifugation. Following this procedure a known potent serum can be rendered inactive. Tables I, II, and III show a marked effect when immune serum A and the parasites are incubated one-half hour at 37°C., a minimal effect when they are not incubated but injected separately, and no effect after absorption of the serum.

There seems to be a stage in the life cycle of the parasite against which an immune serum can be shown to exert its greatest influence. This is upon the mature segmenting form shortly before it sporulates. The enhanced effect on the segmenting forms can be readily seen when the same serum is tested for its capacity to protect against an equal number of young rings, although the segmenting forms divide rapidly so as to increase the number of parasites approximately sixteen fold. This probably indicates that the developing parasite is protected until by its own growth it damages the red cell and thus exposes itself to the action of the circulating immune substances. It is realized that some phagocytosis of the earliest parasitized red cell or even the normal red cell is constantly taking place especially when the macrophages are very active. Nev-

TABLE I

Incubation of Immune Serum A with 10,000 P. knowlesi Parasites ½ Hour at 37°C. before Intraperitoneal Injection

MONKEY NUMBER	AMOUNT OF SERUM A	NUMBER OF PARASITES	DAY PARASITES APPEARED IN BLOOD STREAM	RESULTS
	cc.			
1	2.0	10,000		No infection
2	0.2	10,000	11	Survived
3	0.02	10,000	9	Died on 18th day
4	0.002	10,000	6	Died on 12th day
Normal serum controls				
5	2.0	10,000	5	Died on 11th day
6	0.002	10,000	6	Died on 12th day

TABLE II

Immune Serum A and 10,000 P. knowlesi Parasites Injected Simultaneously at Different Sites

MONKEY NUMBER	AMOUNT OF SERUM A	NUMBER OF PARASITES	DAY PARASITES APPEARED IN BLOOD STREAM	RESULTS
	cc.			
7	2.0	10,000	8	Severe infection; recovered
8	0.2	10,000	6	Died on 12th day
9	0.02	10,000	5	Died on 13th day
10	0.002	10,000	5	Died on 9th day
Normal serum controls				
11	2.0	10,000	6	Died on 11th day
12	0.02	10,000	5	Died on 10th day

TABLE III

Incubation of Absorbed Serum A with 10,000 P. knowlesi Parasites ½ Hour at 37°C.

MONKEY NUMBER	AMOUNT OF SERUM A	NUMBER OF PARASITES	DAY PARASITES APPEARED IN BLOOD STREAM	RESULTS
	cc.			
13	2.0	10,000	6	Died on 14th day
14	0.2	10,000	6	Died on 13th day
15	0.02	10,000	5	Died on 12th day
16	0.002	10,000	5	Died on 10th day
Normal serum controls				
17	0.2	10,000	5	Died on 10th day
18	0.002	10,000	6	Died on 11th day

ertheless the majority of the parasites are probably removed from the circulation during the vulnerable period when they reach maturity and are released from the cell. These and other similar findings indicate that a malarial immune serum can act upon the parasite in the absence of fixed tissue cells and that the humoral and cellular elements do not operate independently but rely upon one another in their defensive efforts. The most conclusive evidence of the combined action was demonstrated by Mulligan and his coworkers who have recently shown that after receiving homologous immune serum *sinicus* and *rhesus* monkeys are less susceptible to *Plasmodium knowlesi* infections if they had also been previously stimulated by the milder *Plasmodium inui* infections (30).

ACTIVE IMMUNIZATION WITH LIVING AND KILLED PARASITES

One of the important weapons in preventive medicine is a successful vaccine, either living or dead. There are no recognized immunological responses to the malarial parasite which lead one to believe that a sufficiently broad immuinty could be induced by infection with any single parasite, whether it be mild or virulent. It has not been possible to demonstrate conclusively the attenuation in virulence of any malarial parasite. In Puerto Rico and elsewhere where the natural habitats of the three human malarial plasmodia overlap, certain individuals may show consecutive infections with each one, indicating a total lack of effective cross immunity (Earle, 31). Even more convincing are the studies mentioned earlier where it was found that an infection only immunized an individual against the local strain of the same species. Except for the very unlikely possibility that a new strain of malarial parasite with exceedingly broad antigenic immunizing complexes should be discovered, there is little hope for an effective living vaccine in malaria.

The use of inactivated parasites as immunizing agents promises no more. For example, attempted immunizations of rhesus monkeys over long periods of time by inoculations with huge doses of *Plasmodium knowlesi* parasites killed by heat, formalin, drying and freezing and thawing does not stimulate a sufficiently active immunity to protect the animals from death when subsequently inoculated with a few living parasites (Eaton and Coggeshall, 32). Recently Gingrich (33) has shown that by injecting canaries intravenously with large numbers of formolized *Plasmodium cathemerium* trophozoites, a partial immunity is obtained and Redmond (34) has reported the same effect with irradiated parasites but these reactions were barely demonstrable and do not approach practicable possibilities. Mulligan and Russell (35) inactivated *Plasmodium gallinaceum* sporozoites by ultra violet radiation, injected them into chickens and found that approximately 50 per cent were partially immunized. The Sergents (36) also claimed partial immunization with attenuated sporozoites. Many other similar attempts have not been attended by any marked success so that one is forced to conclude that the acquisition of immunity following the inoculation of killed malarial organisms is only demonstrable under exceptional conditions.

EVIDENCE OF OTHER MALARIAL ANTIBODIES

Following the demonstration of specific protective antibodies, the characteristic behavior of *Plasmodium knowlesi* in the rhesus monkeys suggested that other types of antibodies might be identified. A complement fixation reaction for malaria had given both positive and negative results in the hands of several workers (reviewed in Coggeshall and Eaton, 37). The chief difficulty encountered was the lack of any means of obtaining a satisfactory supply of antigenic material as the usual source was either infected placentas or post mortem spleens. Since the *Plasmodium knowlesi* infections in rhesus monkeys provide such an abundant supply of parasites, this handicap was overcome. By the simple process of freezing and thawing the infected cells and making a saline extract it was possible to obtain a very efficient antigen. It was frequently possible to obtain from a single rhesus monkey as much as one liter of antigen that could be relied upon regularly to detect complement fixing antibodies in dilutions of serum of 1:32 or higher. Observations on the acquisition and behavior of the complement fixing antibodies showed that in monkeys the titre rapidly rises after the initial attack, then gradually declines and seeks a level characteristic for the individual monkey. The level is maintained except immediately preceding and following successive relapses when the concentration falls and then rises as does the protective antibody. A high titre of these antibodies generally is associated with a high protective antibody level although in individual animals the reverse situation may prevail. It is frequently possible to predict the occurrence of a relapse in advance of the reappearance of parasites in the blood stream by a sharp drop in the titre of complement fixing antibodies as was noted with the protective antibody.

In evaluating the specificity of the complement fixation reaction it was gratifying to learn that it was group specific but not species specific. The antigenic range was so broad that it would bind antibody produced by any of the human infections as readily as that produced by the homologous organism in monkeys or in *Plasmodium knowlesi* infection in man. This finding of group specificity suggested a practical application because an improved method of diagnosis is recognized as an outstanding need in malaria particularly in those low grade infections or treated cases where the parasites are so scanty that they escape microscopic detection. It would only assume importance, however, in such cases because there is no more positive finding than the detection of the parasite. Recent unpublished studies have shown that the reaction will remain positive for some months after it is no longer possible to make a blood smear diagnosis. Its real evaluation, however, must come from areas where malaria is endemic and some preliminary studies are encouraging (Stratman-Thomas, 38, and Dulaney and Kligler, 39). These findings confirm and extend the observations of earlier workers and at the same time add more evidence in support of the thesis that the immune response to the malarial parasite is similar to those observed in other infectious diseases.

AGGLUTINATION OF MALARIAL PARASITES

The specific agglutination of a malarial parasite *in vitro* was also first observed with *Plasmodium knowlesi* in 1938 (Eaton, 40) and this observation has since been confirmed by others (Singh and Singh, 41, and Somogyi, 42). Recently the specific agglutination of viable and inactivated sporozoites of *Plasmodium gallinaceum* has also been observed (Mulligan, Russell, and Mohan, 43).

In a *Plasmodium knowlesi* infection the agglutinins appear about the time the host is able to maintain control of the infection without the aid of chemotherapy or immune serum. The agglutinating titre of the serum is very low at this stage but becomes progressively higher with the development of immunity, often being positive in dilutions of 1:1000. Mulligan, Russell and Mohan (43) have noted specific agglutination with *Plasmodium gallinaceum* sporozoites in serum diluted 1:262,144. Cross reactions with other parasites are negative so that the agglutinin like the protective antibody is species specific and thus differs from the group specific complement fixing antibodies.

One of the most interesting features of the reaction is that the red cells containing the immature ring forms do not agglutinate but only the fully developed segmenting forms. This again indicates a direct action between the antigen and antibody and is in agreement with the greater protective effect obtained with immune serum against the more mature parasite in the protection test. Since the agglutination reaction is demonstrable *in vitro* it also suggests that a specific sensitization could occur *in vivo* and that the phagocytic cells of the host could more readily remove the "clumped" parasitized erythrocytes. Proof of this assumption does not necessarily depend upon the direct observation of auto-agglutination in the peripheral blood but a few such instances have been reported both with *Plasmodium gallinaceum* and *Plasmodium knowlesi* (Malamos, 44). It is not possible to define the exact rôle of the agglutinating antibody but the initial activity of certain humoral immune substances such as the agglutinins may determine the success of the host in defending itself against the invasiveness of the parasite.

DURATION OF IMMUNITY

The material thus far presented has furnished experimental evidence of the acquisition, identity and characteristic behaviors of certain humoral immune substances in malaria that emphasize their essential rôle in the development of the immunity mechanism in the host. It also has been pointed out that the combined defensive efforts of the humoral and cellular elements are highly effective within certain recognized limitations for a considerable period of time but thus far neither these nor other studies have shown whether the host is resistant because it possesses a residual immunity following the complete disappearance of an infection or if it is immune by reason of the fact that it harbors an undetectable infection.

The introduction of therapeutic malaria into non-malarial areas provided a means of obtaining valuable information under controlled conditions with pa-

tients inoculated by the bite of the mosquito. The results of the observations on malarial immunity in man from many different places show that many patients acquire a high degree of species-specific and probably strain-specific immunity that can persist for many years. The possibility of a subclinical, submicroscopic infection is quite possible because there are many authenticated instances where individuals have transmitted malaria by blood transfusions to a susceptible recipient as long as thirty-five years following their initial attack, having been asymptomatic in the interim and living in areas where the transmission of malaria does not occur. Investigations on the behavior of *Plasmodium knowlesi* infections in man clearly demonstrate the difficulty of detecting known infections and the limited value of the negative blood smear. Thick and thin preparations from one patient were examined daily for 114 days and no parasites were detected, yet when his blood was subinoculated into a susceptible rhesus monkey it was shown to contain viable parasites. In this same study it was also observed that it was possible for a negro patient to become infected following inoculation with *Plasmodium knowlesi* parasites without the development of clinical symptoms or microscopically visible parasites. This patient's blood was infectious for rhesus monkeys on the second, fourth, tenth, and eighteenth day. There was a consistent multiplication of the parasites in the presumably normal patient (Milam and Kusch, 45).

In naturally acquired human malaria there are three main reasons why it is almost impossible to know the status or duration of immunity. First, in areas where malaria is prevalent usually two if not all three of the human infections may be present, appearing consecutively in the same individual. Also it is not possible to differentiate a relapse from a reinfection. Finally, since neither quinine nor atebrin eradicates the parasites but merely converts an active infection into a latent one, there is no means of determining precisely when a complete cure takes place. These and other examples furnish more than presumptive evidence of our inability to define the exact limits of a malarial infection.

Considerable aid was given to the study of this problem when it was found possible completely to eradicate an acute or chronic *Plasmodium knowlesi* infection in the rhesus monkey by giving a single dose of sulfanilamide by mouth. Proof that this procedure would sterilize the infection promptly was the inability to produce an infection by subinoculation with massive amounts of the treated animal's blood although as previously stated less than ten parasites constitute a minimal infective dose; the failure to evoke a relapse by splenectomy; the noninfectiousness of the emulsified spleen and finally susceptibility to reinfection with the same organism. Quinine or atebrin are unable to accomplish this effect even when given up to their toxic limits over long periods.

If an animal's infection is interrupted during its early stages before the invasive activity of the parasite and the defensive forces of the host have reached a state of equilibrium, the resultant immunity is so meager that when reinoculated a few days later the animal is not protected against fatal infection.

With very little stretch of the imagination one can discern the limitations and potentialities of artificial immunization as a means of preventing or controlling

malaria in this one experiment. Thus when an infection so severe that death of the host can be predicted within a few hours, fails to produce an immunity sufficient to last more than a few days, it is quite evident that one should not expect to immunize normals by the injection of a relatively few inactivated parasites prepared in any type of a vaccine.

A different picture presents itself when one converts an acute infection into a chronic one by quinine, atebrin, or immune serum and destroys this infection with a sulfonamide drug. A resultant immunity may be demonstrated which is partially effective for approximately six months. Animals so treated will acquire severe infections upon reinoculations but all will survive. Contrary to expectations, the degree of immunity following eradication of the infection does not seem to depend upon the duration of the infection previous to its destruction. These preliminary experiments disprove the thesis postulated by many that immunity to malarial infection terminates immediately upon the disappearance of the infection and it is evident that there is some residual immunity following eradication of the infection.

It is impossible to state at present what factors determine the exact duration of this immunity, but one may speculate that it is the time necessary for the defensive cells of the host to assimilate completely the concentrated parasites and pigment so that they no longer serve as a source of material to stimulate antibody production.

The ability of sulfonamide drugs now in current use to eradicate *Plasmodium knowlesi* infections has an implication in the problem of human malaria. Although thus far none has been discovered to be as efficient against the human infections, the possibility of such a discovery is not remote. If one should be found it will probably also serve as a true causal prophylactic, something entirely lacking at present.

SUMMARY

In the foregoing discussion an effort was made to explain certain manifestations of malarial immunity in the light of recent investigations which have been conducted in man and animal. The major emphasis was directed to those studies in which it was found possible to demonstrate the existence and certain behaviors of some of the circulating antibodies acquired during the development of immunity. It was pointed out that their rôle in the defense mechanism was not an insignificant one but highly essential for the protection of the host. Experimental evidence suggests that the protective substances in the immune serum sensitize the parasite and thus make it more vulnerable to phagocytosis and that the effective functioning of the defensive efforts of the host does not depend solely upon a cellular or humoral basis but upon the cooperative efforts of both.

It becomes quite apparent that the characteristic behavior of the malarial antibodies is similar to that observed in the immunological reactions of any host attempting to defend itself against pathogenic organisms. The detection of these antibodies together with an understanding of their specific reactions pro-

vides a means whereby the different aspects of the malaria problem can be re-examined.

Analysis of the data from the standpoint of defining the status of immunity to the malarial parasite in its broadest sense shows that it is essentially an infection immunity. It has been possible to show that latent malaria in experimental animals can be completely destroyed by chemotherapy and that the duration of immunity thereafter is brief, usually a matter of months, while the eradication of an acute infection in its earlier stages leaves the host with no immunity whatever. On the basis of these two findings alone, especially as regards the infection in the host, it seems that one can anticipate the direction of productive research for the future.

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STUDIES OF CALCIUM AND PHOSPHORUS METABOLISM WITH SPECIAL REFERENCE TO PATHOGENESIS AND EFFECTS OF DIHYDROTACHYSTEROL (A T 10) AND IRON

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NOSOGRAPHY

The purpose of this presentation is to record the metabolic observations on five cases of renal osteodystrophy in the hope that they may contribute to the understanding of the pathogenesis of this osseous disorder and add to its therapy The term renal osteodystrophy is chosen advisedly, because while chronic renal insufficiency is fundamentally responsible for the skeletal changes in all the cases, the type of resulting osseous disorder is not uniform and may be difficult to determine without complete histological examination Moreover in the literature the nosography of the disease is a subject of much controversy The term "renal rickets", though used extensively, is objectionable because it identifies the disease of the skeleton with rickets which it is not in many cases, especially those in more recent reports, although the radiological appearance and the gross deformities of the bones resemble those seen in rickets The term "renal dwarfism" emphasizes only one feature of the disease which is not present in many cases especially when growth is complete and fails to indicate the osseous affection Objection to the term "renal infantilism" may be made on the same basis In view of the finding of osteitis or osteoporosis fibrosa cystica in many cases in which the pathologic study was clearly presented, Park and Eliot (1) inclined to consider osteofibrosis as the essential pathological lesion in the skeleton and agreed to the propriety of the term "renal osteitis fibrosa cystica" adopted by Albright, Drake and Sulkowitch (2) How generally applicable this term may be will have to await the pathological studies of further cases The term "renal osteodystrophy" seems to be a suitable generic name to include cases of

osseous disorder associated with renal insufficiency, while the exact nature of the pathological process in the skeleton is still undetermined.

HISTORICAL

The association of albuminuria and late rickets was noted as early as 1883 by Lucas (3), but it was not until 1911 that Fletcher (4) clearly recognized the etiologic relationship between chronic renal disease and bone deformities. Barber (5) reported 10 additional cases under the term renal dwarfism. Parsons (6) and Teall (7) are early workers to define the roentgenologic picture of renal rickets. Broekman (8) gave the first clear histological distinction between renal rickets and infantile rickets. Recent reports by Langmead and Orr (9), Smyth and Goldman (10), Shelling and Remsen (11), Albright, Drake and Sulkowitch (2), and others (12–14) served to emphasize the frequent occurrence of diffuse parathyroid hyperplasia in renal osteodystrophy and raise the question of its possible etiologic importance in the genesis of the osseous disorder. Comprehensive reviews of the subject of renal osteodystrophy are available by Mitchell (15), Hamperl and Wallis (16) and Park and Eliot (1).

CLINICAL MATERIAL

The present study includes 5 Chinese patients with various grades of skeletal decalcification with or without other osseous changes occurring in association with moderate to advanced renal insufficiency. The clinical abstracts of these patients are to be found in the appendix. Here the salient features of each case may be briefly summarized.

Case 1 concerns a girl of 8 who had scarlet fever and acute glomerulonephritis 2 years previously. This was followed by polyuria, pain in legs and stunted growth. Examination showed dwarfism, knock-knee, enlarged wrists and ankles. X-ray revealed marked rarefaction of the skeleton with rachitis-like changes in the epiphyses. There was slight albuminuria with moderate reduction of renal function.

Case 2 is that of a girl of 19 who developed knock-knee, pain in the legs, pelvis and spine and great debility in the course of 4 years. The skeleton showed general osteoporosis and marked deformities. The pelvis exhibited cystic absorption and a triradiate deformity of the type seen in osteomalacia. The epiphyses had already been fused. Moderate anemia, slight albuminuria and very poor renal function were present. Pyelogram showed very small kidney shadows.

Case 3, a married woman of 34, was admitted in 1938 during her sixth pregnancy for edema, bone pains and spasm of hands. She had similar, but milder, symptoms in her fourth and fifth pregnancies 4 and 2 years previously. Bleeding from hemorrhoids had been present for 7 years. Examination showed severe anemia, moderate cardiac enlargement and slight peripheral edema. Signs of tetany were present. There was bone tenderness, but no deformities. X-ray showed slight rarefaction of all the bones with biconcave reabsorption of the lumbar vertebrae. Renal functional impairment was severe, and pyelography revealed very small kidneys. Slight albuminuria was present.

Case 4. This is a girl of 20 who had generalized bone aching for over a year. There was slight or moderate tenderness over the bones of the lower extremities, pelvis and chest, but no deformities. Generalized moderate osteoporosis was demonstrated on x-ray examination. There were moderately severe anemia and slight albuminuria. The renal function was markedly impaired and the kidneys appeared to be very small on the pyelogram.

Case 5 is that of a boy of 20 with pain and swelling of knees, progressive deformity of lower limbs and backache for 8 years. Examination showed emaciation, dwarfism and infantilism. Both knees were swollen and tender with effusion. The bones showed extreme rarefaction. The left femur presented an old fracture. The diaphyseal ends of both radii showed marked cupping and irregularity. The skull presented a mottled appearance and the vault was thinner than normal. The urine contained albumin and the renal function was very poor. Moderate anemia was present.

The bones of all the five patients were involved to a varying extent. Slight or moderate osteoporosis without skeletal deformity was present in Cases 3 and 4, and marked osteoporosis in the rest of the cases. In addition, Case 2 showed deformities of the pelvis and knees with cystic absorption of some of the bones. Cases 1 and 5 presented rachitis-like changes in the epiphyses. In the last case rheumatoid arthritis might also be present. The nature of the kidney disease was not determined in any of the cases. In Case 1, the history of acute nephritis following scarlet fever suggested chronic glomerulonephritis (16a). In the remaining cases there was no history referable to renal disease, and the advanced renal insufficiency was discovered on renal function tests. The small kidneys demonstrated on the pyelograms could be the result of chronic glomerulonephritis or pyelonephritis, or they were congenitally hypoplastic with interstitial nephritis.

METABOLIC PROCEDURE

All the patients were observed for prolonged periods in the metabolism ward where the procedure for making and serving constant diets and quantitative collection of excreta has been established and described previously (17). The diets for these patients were usually low in calcium and phosphorus (Table 6, Appendix). The desired high level of calcium intake was made up by the administration of a 7.7% calcium lactate solution. Higher phosphorus intake was brought about either by inclusion in the diet of phosphorus-rich food or by the administration of a mixture of monosodium and disodium phosphate of pH 7.40, disodium or trisodium phosphate solution. The diets were quantitatively consumed except in a few instances where the refused food or vomitus were saved and analysed. Complete balances in calcium, phosphorus and nitrogen were obtained in 4-day periods. Serum calcium, phosphorus and phosphatase (18) were determined at the beginning of each period. The massed data on the 5 patients are shown in Tables 7–12 in the Appendix.

Serum acid-base balance from venous blood obtained with anaërobic technic was studied occasionally in each patient and more frequently when the influence

of ingestion of sodium bicarbonate or ammonium chloride was investigated. The acid-base studies included serum pH (19), bicarbonate (20), chloride (21), inorganic phosphate (22), proteins (23), total base (24) sodium (25), potassium (26), calcium (27) and magnesium (28).

Vitamin D given orally was an oily solution of irradiated ergosterol marketed as Vigantol containing 0.3 mg. or 12,000 international units per cc. or 30 drops. For intramuscular injection Vigantol was sterilized by autoclaving at 15 pounds of pressure for half an hour. A concentrated solution of irradiated ergosterol in corn oil[1] containing 1,000,000 international units per gm. was given orally for the single massive dose therapy. A.T.10, given by mouth was a 0.5% solution in oil of dihydrotachysterol (Bayer). For the oral administration of iron, a 20% solution of ferric ammonium citrate was used.

ALTERED PHOSPHORUS METABOLISM IN RENAL OSTEODYSTROPHY

The fundamental cause of renal osteodystrophy must be sought in the renal insufficiency of long standing. The duration of the renal disease in our cases can not be accurately ascertained, but probably it ranged between 2 and 8 years. The severity of the renal failure must also play a part, for in 4 of the cases in this series, the phenolsulphonephthalein output was only 6% or less, and the urea clearance varied around 10 per cent of normal, and the azotemia was pronounced. However, advanced renal insufficiency is not an invariable accompaniment of renal osteodystrophy. Case 1 showed only moderate renal insufficiency, although the osseous involvement was severe. While it is generally agreed that renal insufficiency is causally related to the development of osseous disorder, much remains to be elucidated as to the mechanism by which the osseous disorder is brought about by the renal insufficiency. Several theories have been proposed.

Mitchell's concept (15) is that in renal insufficiency a shift of phosphorus excretion from the kidneys to the intestine occurs, and "the concentration of phosphorus in the intestine thus increased so blocks the absorption of calcium from food that the child suffers a true calcium starvation." Although the theory has been accepted generally and received support from Albright, Drake and Sulkowitch (2), the experimental data demonstrating the shift of phosphorus excretion and impairment of intestinal absorption of calcium in renal osteodystrophy remain meager. The presence of chronic acidosis in renal insufficiency has been thought by many to be an important factor in the skeletal decalcification in renal osteodystrophy. In normal individuals (29) and in patients with osteomalacia (30) the production of moderate acidosis by the ingestion of ammonium chloride results in excessive calcium wastage through the urinary tract. Jaffe, Bodansky and Chandler (31) produced in animals a bone disorder similar to osteitis fibrosa by feeding them a low calcium diet and ammonium chloride. Shohl (32) found rickets in the rat fed a non-rachitogenic diet with added ammonium chloride and ammonium carbonate mixture. While acidosis does exert a deleterious effect on the calcium and phosphorus metabo-

[1] Supplied through the kindness of Dr. Charles E. Bills of the Research Laboratory, Mead Johnson and Company, Evansville, Ind.

ism, how important it is in the pathogenesis of renal osteodystrophy remains to be determined. Recently a third theory has been put forward, namely, that hyperparathyroidism secondary to renal insufficiency occurs and is the cause of the osseous changes. While this concept is propounded in detail by Park and Eliot (1) and Anderson (33), it has been objected to by others, notably Albright, Drake and Sulkowitch (2).

The data from the present series of cases concerning the disturbances in phosphorus metabolism may be of value in evaluating the various theories of pathogenesis of renal osteodystrophy.

(a) *Serum inorganic phosphorus*

In most of the reported cases of renal rickets the serum inorganic phosphorus was very much elevated. In fact, phosphate retention in the blood has been regarded by many as the *sine qua non* of renal rickets. In our series Cases 2, 3 and 4 showed initial values averaging 5.90, 6.89 and 4.76 mg.% respectively indicating definite phosphate retention (Table 1). Cases 1 and 5 had values around 4 mg.%, that is, within normal limits. As the renal functional impairment in Case 1 was only moderately severe, definite elevation of serum inorganic phosphorus may not be expected. However, we have no explanation to offer for the lack of phosphate retention in Case 5. The renal insufficiency of this patient was just as severe as that of the other patients in this series with definite elevation of serum inorganic phosphorus. We suspect that the relatively low phosphorus diet might have lowered the serum inorganic phosphorus. In fact, when these patients were examined in the outpatient clinic, their serum phosphorus was frequently lower than after admission, suggesting that the diet at home had been lower in phosphorus content or contained largely cereal phosphorus which could not be absorbed. Then there is the possibility of variations in the response of the parathyroids to renal insufficiency. An active response of the parathyroids may depress an otherwise elevated serum inorganic phosphorus to normal, while a relative inactivity of the parathyroids favors the development of hyperphosphatemia. Thus, while hyperphosphatemia is the rule in advanced renal insufficiency, exceptions do occur in view of certain modifying factors such as low phosphorus diet and activity of the parathyroids.

(b) *The ratio of urine to stool excretion of phosphorus*

Mitchell (15) rearranged the data of Boyd, Courtney and MacLachlan (34) on 7 cases of chronic nephritis and those of Schoenthal (35) on 1 case of renal rickets and 2 cases of chronic nephritis for comparison with the data on 4 normal children as controls. The ratio of urinary to fecal phosphorus in the controls varied from 1.02 to 2.01, while that in the nephritic children was below 1 in 6 out of 10 cases, and above 1 in the remainder. This was interpreted to indicate a shift of phosphorus excretion from the kidneys to the intestine in renal insufficiency. However, the partition of phosphorus excretion depends to a large extent on the relative proportion of calcium and phosphorus in the diet. When more calcium is ingested in relation to phosphorus (high Ca/P intake), more

phosphorus is excreted in the feces than in the urine; and when the diet contains less calcium in proportion to phosphorus (low Ca/P intake), phosphorus excretion takes place more in the urine than in the stool. This is strikingly shown in osteomalacia undergoing repair through the action of vitamin D (36, 37). The same phenomenon to a lesser degree is also observed in normal individuals (38). Therefore the urinary and stool phosphorus data need to be interpreted in conjunction with the ratio of calcium to phosphorus intake. In the collected cases of Mitchell, the diet Ca/P ratio was high, ranging from 0.87 to 2.32, favoring high phosphorus output in the stool. Therefore, in those cases in which the ratio of urinary to fecal phosphorus was below 1, such effects could not be entirely attributed to renal insufficiency.

In Table 1 are set forth the percentage of phosphorus excretion in the urine in terms of total output in the present series of cases when the diet Ca/P intake was low, namely 0.27–0.44. In case 1, the urinary phosphorus amounted to 76% of the total output, a figure at the upper limit of normal, evidently due to the fact that her renal function was not sufficiently impaired to influence the partition of phosphorus excretion. In the remaining 4 cases where the renal insufficiency was advanced the urinary phosphorus varied from 40 to 54%, all below the average of 63% for normal individuals on similar ratio of calcium to phosphorus intake. This indicates considerable difficulty on the part of the kidneys to eliminate phosphorus under the strain of a low Ca/P intake. When the phosphorus intake remained about the same, but with the calcium intake raised so as to increase the diet ratio of Ca/P to 1.80–2.63, all showed a definite decrease in the percentage of phosphorus excretion in the urine, demonstrating the influence of a high calcium intake in diverting phosphorus excretion from the kidneys to the intestine. In general the decrease in urinary phosphorus percentage was more pronounced in advanced renal insufficiency than in normal persons and in the case of milder renal disease, suggesting that in the former the strain on the kidneys of a low Ca/P intake had been greater.

(c) *Effect of increasing the phosphorus intake*

Attempt was made in examining further the metabolic behavior of these patients when the phosphorus intake was raised. Under the stress of a high phosphorus intake, one expects to find phosphorus retention manifested not only by an elevation of serum inorganic phosphorus but also by a positive balance or an increase of such. The large amount of ingested phosphorus should give rise to greater increase of phosphorus output in the stool than in the urine, thus reducing still further the percentage of phosphorus output in the urine. If the stool phosphorus is sufficiently augmented, the stool calcium may be expected to increase, thus diminishing calcium retention or rendering a positive calcium balance negative. All these expectations come true to a varying extent in the four cases so studied.

Case 2. As shown in Fig. 1, periods 6 and 7 were control periods on an intake of 1207 mg. calcium and 675 mg. phosphorus. On this regime the serum phosphorus was coming down from 6.85 to 5.96 mg.%, and the serum calcium

was essentially constant at 5.54–5.91 mg.%. Urinary phosphorus averaged 314 mg. per day, amounting to 43% of the total output; and the balance was on the average −56 mg. per day. The average daily calcium balance was 63 mg., the large output being all by way of the intestine. During the next two periods (periods 8 and 9), the phosphorus intake was increased to 1075 mg. per day by the administration of a solution of disodium phosphate. This was followed by a rise of serum inorganic phosphorus from 5.96 to 7.25 mg.% and by a fall of serum calcium from 5.54 to 4.86 mg.%. Clinically the patient felt worse and exhibited active tetany. The urine phosphorus increased to 418 mg. daily, namely, 44% of the total output, and the balance was changed to a positive one, namely, 117 mg. per day. With this phosphorus gain there was a slight improvement of calcium balance to 114 mg. per day. The next two periods (periods 10

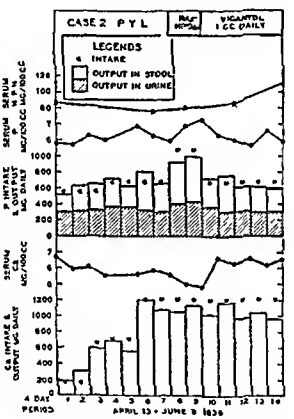

FIG. 1. CASE 2. EFFECT OF HIGH PHOSPHORUS INTAKE AND VIGANTOL ON CALCIUM AND PHOSPHORUS METABOLISM

and 11) saw a reversion of most of the alterations to the control state. It is noteworthy that during these two periods the phosphorus balance was a little more negative than in the control periods suggesting that the retained phosphorus during the high phosphorus periods was now being slowly eliminated.

Case 3. While on a daily calcium intake of 989 mg. this patient had her phosphorus intake raised from 612 mg. (periods 16–17) to 1105 mg. (period 18) and 1055 mg. (period 19) by the addition of a phosphate solution. Such a change resulted in a marked increase of the serum-inorganic phosphorus from 5.58 to 9.26 mg.% and a decrease of the serum calcium from 5.46 to 4.14 mg.% (Fig. 2). The daily urine phosphorus excretion increased from 218 to 323 mg. but the proportion in the urine increased but little (from 33 to 36%). The phosphorus balance shifted from −40 to 176 mg. per day, while the calcium balance changed from 31 to −12 indicating that an increase of stool phosphorus also increased

stool calcium, leading to an adverse calcium balance. The figures for the following period (period 20) are most instructive. Although the diet phosphorus was lowered to only 585 mg. per day, the stool phosphorus of 684 mg. was in excess of the intake and this, together with the urine output of 257 mg., resulted in a negative balance of 356 mg., showing that the phosphorus retained during the 2 high phosphorus intake periods was eventually all excreted in the following low phosphorus period. With the large increase of stool phosphorus in an attempt to excrete the retained phosphorus, there was a corresponding increase of stool calcium, giving rise to a negative balance of 295 mg. per day. This set

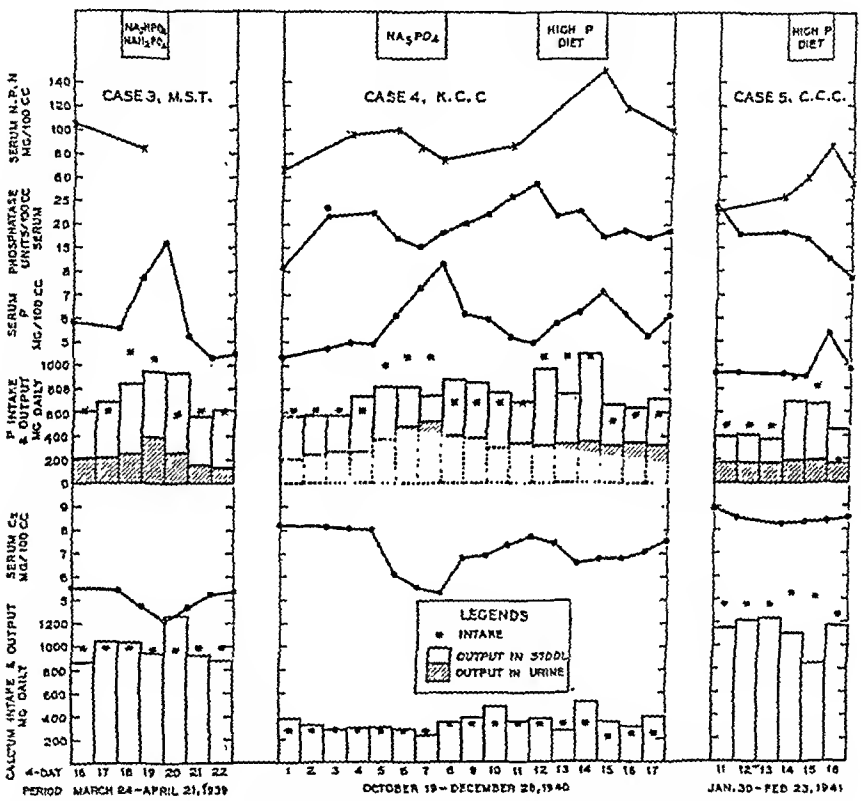

FIG. 2. CASES 3, 4 AND 5. EFFECT OF HIGH PHOSPHORUS INTAKE ON CALCIUM AND PHOSPHORUS METABOLISM

of data affords direct evidence to the supposition that phosphorus elimination by the bowel in large amounts does lead to a loss of calcium, and this loss continues for sometime after the phosphorus intake is reduced.

Case 4. In this patient the effect of increasing phosphorus intake was studied while on low calcium diets (215–336 mg. per day), and the phosphorus intake was raised by the addition of trisodium phosphate in the first series (periods 5–7) and by the inclusion in the diet of high phosphorus-containing foods such as millet, peanut and pork liver in the second series (periods 12–14). Periods 1–4 served as control on a diet containing 271 mg. calcium and 618 mg. phosphorus (Fig. 2). On this diet the calcium balance was slightly negative, averaging 52 mg. per day and the phosphorus balance was barely positive, averaging 11 mg.

per day. The output of calcium was practically all in the stool, and the partition of phosphorus excretion was 40% in the urine. The serum calcium tended to lower, while inorganic phosphorus tended to rise. When the phosphorus intake was raised to 1059 mg. per day by the addition of trisodium phosphate, there was a marked phosphorus retention, averaging 256 mg. per day, and the serum inorganic phosphorus rose from 4.95 to 8.33 mg.%, with a lowering of the serum calcium from 8.04 to 5.38 mg.%. However, in this patient the increase of phosphorus intake by the addition of trisodium phosphate did not increase the stool phosphorus, showing that the phosphate in this form was easily absorbed. The urinary phosphorus increased, amounting to 58% of the total output, indicating that the kidneys were still capable of bearing most of the burden of phosphorus excretion. With the absorption of relatively large amounts of phosphate, the calcium balance showed a slightly favorable turn, the negative balance now averaging only 9 mg. per day. The post-control periods (periods 8–11) without the added phosphate witnessed an increase of stool phosphorus excretion over the pre-control periods (periods 1–4) with a persistently negative balance averaging 122 mg. per day. The serum inorganic phosphorus steadily returned to 5.00 mg.%, and the serum calcium to 7.73 mg.%. The calcium balance became less favorable, averaging −60 mg. per day.

During periods 12–14 the diet was changed to contain 1073 mg. of phosphorus. On this relatively high phosphorus intake of exclusively dietary origin, there was likewise a retention of phosphorus, averaging 124 mg. per day, and an increase of the serum inorganic phosphorus from 5.00 to 7.11 mg.% with a decrease of the serum calcium from 7.73 to 6.80 mg.%. These changes were not as marked as those of periods 5–7 when a similarly high phosphorus intake was brought about by the administration of sodium phosphate. The explanation for the difference probably lies in the poorer absorbability of the dietary phosphorus, particularly cereal and nut phosphorus. Thus the stool phorphorus during periods 12–14 showed a marked increase averaging 610 mg. per day and the urinary phosphorus came up to only 37% of the total output. The calcium balance remained negative to about the same extent as previously, namely, −62 mg. daily. In the three post-control periods (periods 15–17) with the diet changed back to a low phosphorus one, the reverse train of events followed: a decrease of the serum inorganic phosphorus and an increase of the serum calcium, a persistence of relatively large stool phosphorus excretion in spite of decreased intake, resulting in a negative phosphorus balance, and a large stool calcium excretion leading to a calcium loss.

Comparing the effects of increasing phosphorus intake by means of the soluble salt of phosphate with those of high phosphorus containing diet one finds that the former was more easily absorbed giving rise to greater retention and, in the serum, greater changes in the levels of inorganic phosphorus and calcium, but the partition of phosphate excretion was more in favor of the urinary tract leading to less phosphorus, therefore less calcium, drainage through the bowel. Clinically the patient tolerated the soluble phosphate salt better than the organic dietary phosphorus, not from the slightly greater drainage of calcium in the

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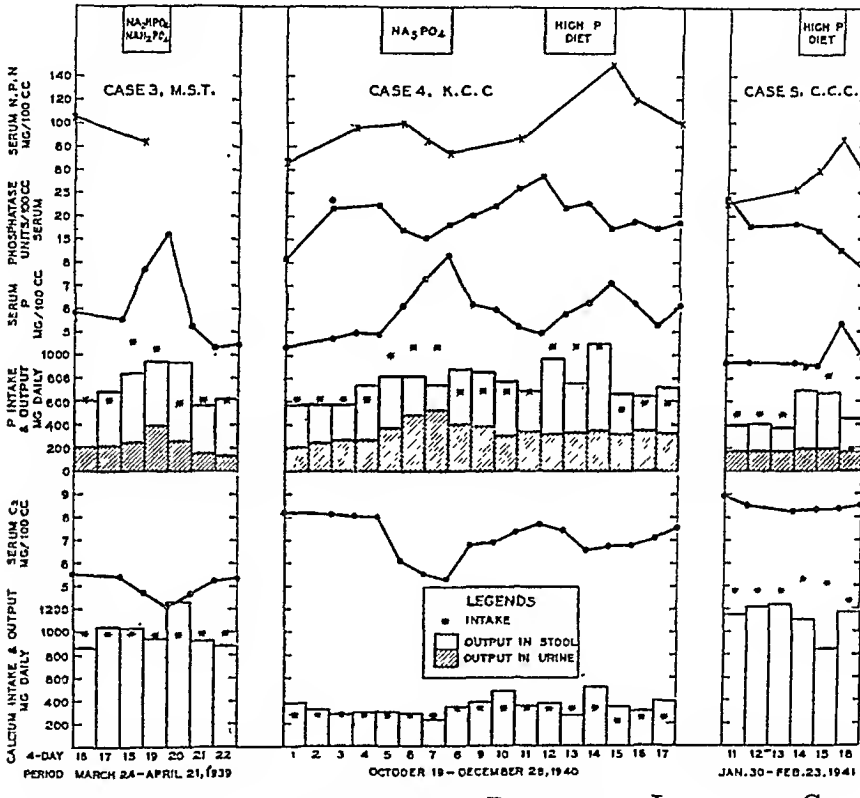

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phosphorus elimination then gave rise to a considerable negative calcium balance in most instances. This calcium loss brought about by the necessity of large stool elimination of phosphorus sheds an important light on the mechanism of skeletal decalcification in renal insufficiency.

This is in contrast to the studies on Farquharson and Salter and their coworkers (39, 40) on individuals with normal kidneys. The ingestion of large amounts of soluble phosphates was followed by prompt elimination. Three-fourths of the excess phosphorus excretion was urinary. The calcium balance was not influenced by the administration of phosphates.

IMPORTANCE OF CALCIUM INTAKE

The usual Chinese dietary is low in calcium and various estimates place the calcium intake at 200–400 mg. per day. Such a level of intake may be sufficient for a normal person when the calcium-conserving action of vitamin D brought about by an adequate exposure to sunlight is operative, and when extra demands for the mineral such as during pregnancy and lactation are not present. However, as soon as sunlight exposure is curtailed by prolonged indoor life and repeated reproductive cycles set in, the prevailing low calcium intake is no longer adequate to prevent the mineral drainage from the skeleton. These circumstances afford an explanation for the prevalence of osteomalacia in Chinese women in the northern parts of China (41, 42). It would be of interest to examine cases of renal insufficiency to see if a low calcium diet can be a factor in the development of the osseous decalcification. In Table 3, are set forth the average daily calcium and phosphorus balances, together with serum calcium and inorganic phosphorus observed after from 2 to 4 periods on low calcium diets. These diets contained from 96 to 415 mg. of calcium per day or from 6.4 to 10.9 mg. per kg. of body weight per day. Such levels of calcium intake were most probably not less than what these patients used to take at home especially in view of their poor appetite. Normal Chinese on similar calcium intake (38) may be expected to maintain themselves in balance especially toward the end of several metabolic periods, where physiological adjustment comes into play. On the contrary these patients showed persistently negative calcium balance, averaging from 18 to 87 mg. per day. Such calcium loss, when prolonged for months or years, as it was most likely so in these cases, would inevitably lead to sufficient skeletal decalcification to be evident clinically. Therefore the prevailing low calcium intake in the Chinese diet is an important factor in the pathogenesis of the osseous disorder in renal insufficiency.

One would, then, expect a high calcium intake to be beneficial in such cases. From Table 3, it may be seen that when the calcium intake was raised approximately from 800 mg. in Case 1 to 1900 mg. in Case 3, all the calcium balances became positive. The average daily gain of calcium varied from 63 to 346 mg. It is true that some of these gains were small, but the maintenance of a positive balance for prolonged periods would add substantially to the skeletal store and alleviate the osseous changes.

The favorable influence of a high calcium intake did not stop at the improve-

latter case, but from the concomitant increase of dietary protein leading to greater nitrogen retention and azotemia (blood non-protein nitrogen was raised from 86 to 150 mg.% as the result of increased protein intake).

Case 5. During the control periods (periods 11–13) on a calcium intake of 1356 mg. and a phosphorus intake of 488 mg. per day, both the daily calcium and phorphorus balances were slightly positive, averaging 149 and 92 mg. respectively (Fig. 2). While the calcium output was entirely in the stool, the phosphorus excretion was 44% in the urine. The serum calcium was 8.31 and inorganic phosphorus 3.70 mg.%. Periods 14 and 15 were test periods in which the phosphorus intake was increased to 907 and 819 mg. per day respectively by the inclusion of millet, peanut and more milk in the diet. The stool phosphorus showed a marked increase, while the urinary phosphorus was not much changed in quantity so that it amounted to only 30% of the total output. The phosphorus balance increased to 221 mg. per day, and the serum inorganic phosphorus came up to 5.36 mg.%. There was practically no change in the level of serum calcium, but the calcium balance showed a distinct improvement, averaging 460 mg. per day. In the post-control period (period 16), the serum inorganic phosphorus promptly returned to the precontrol level, the phosphorus balance became markedly negative and there was a corresponding decrease of calcium balance. In this patient the chemical alterations following the increase of diet phosphorus were not as marked as in the previous cases. This is partly due to the poorer general condition of the patient. He tolerated the high phosphorus diet so poorly that he succeeded in finishing the prescribed diet only for the first period (period 14), and the food intake during the subsequent two periods was considerably reduced. The blood non-protein nitrogen increased from 43 to 86 mg.% after two periods of increased nitrogen intake incident to the increase of diet phosphorus.

Summary. As summarized in Table 2, the most marked and consistent changes following an increase of phosphorus intake in these cases of advanced renal insufficiency consisted of a rise of the serum inorganic phosphorus and a retention of phosphorus. This increased intake also led to larger phosphorus output in the stool and somewhat larger excretion in the urine except in Case 4 during the soluble phosphate administration. Here with a ratio of calcium to phosphorus intake as low as 0.26, the kidneys were forced to bear most of the burden of increased phosphate elimination. The serum calcium usually bore an inverse relationship to the serum inorganic phosphorus. Marked lowering of the serum calcium to the tetany level occurred when there was considerable elevation of the serum inorganic phosphorus. The calcium balance was at times improved during the high phosphorus intake periods on account of increased phosphorus retention, but when the stool phosphorus was markedly increased by such a regimen, the calcium balance was usually adversely affected. After the high phosphorus intake was removed during the post-control periods there occurred a rapid elimination of the retained phosphorus largely by way of the bowel, resulting in marked negative phosphorus balance and reversion of the serum inorganic phosphorus and calcium to the control level. The large stool

phosphorus elimination then gave rise to a considerable negative calcium balance in most instances This calcium loss brought about by the necessity of large stool elimination of phosphorus sheds an important light on the mechanism of skeletal decalcification in renal insufficiency

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The favorable influence of a high calcium intake did not stop at the improve-

ment of the calcium balance. The effect on phosphorus balance of a high calcium intake was not uniform, because, on one hand, the increased stool calcium would favor the elimination of phosphorus by the bowel, tending to decrease phosphorus retention, and, on the other hand, better calcium retention might lead to better phosphorus retention for deposition in the bones. The resulting phosphorus balance would, then, be the algebraic sum of the two processes which went on at varying rates in various cases. Thus one finds that the phosphorus balance was improved by high calcium intake in Cases 1 and 4, impoverished in Cases 2 and 3 and unchanged in Case 5.

However varied were the effects of high calcium intake on the phosphorus balance, the response of the serum inorganic phosphorus was most uniform. In all the cases there was a fall of the serum inorganic phosphorus after the high calcium diet. The decrease was most marked in Case 3 (from 7.60 to 4.90 mg.%) and least in Case 2 (from 6.25 to 5.96 mg.%). Even in Cases 1 and 5 where the serum inorganic phosphorus was within normal limits (4.06 and 4.07 mg.% respectively) there was a distinct decrease (to 3.26 mg.% in both cases).

Serum calcium, however, behaved irregularly. There was a distinct increase in 2 instances (Cases 3 and 5), slight decrease in 2 (Cases 1 and 2) and no change in 1 (Case 4).

THE FACTOR OF CHRONIC ACIDOSIS

In renal insufficiency acidosis arises on two scores. First, the retention of phosphate, sulphate and other undetermined acid metabolites takes up base. Second, the conversion of urea into ammonia by the kidneys for the purpose of eliminating acid metabolites as ammonium salts becomes deficient. Thus more fixed base (sodium or potassium) is drawn upon in excreting acid end-products. Both of these processes reduce the amount of fixed base available for the bicarbonate. A decrease of the bicarbonate content tends to decrease the pH of the serum giving rise to acidosis. Chronic acidosis as shown by a decrease of alkali reserve in renal rickets was first noted by Green (43) and confirmed by Lathrop (44), Faxen (45), Elliott (46), Salvesen (47), and almost all recent authors on the subject. Complete serum electrolyte studies are available in some of the reported cases (2, 35, 47). In view of the decalcifying effects of acidosis experimentally produced in man and animals (29–32), it is generally assumed that acidosis is an important factor in the genesis of the osseous disorder in renal insufficiency. The data on the acid-base equilibrium of the serum in the present series of cases and the results of administering alkali and acid to one of the cases lend support to this opinion.

(a) *Serum acid-base balance and electrolytes*

In table 4 are compiled data on serum pH and electrolytes of the cases in this series. The pH was around 7.25–7.30 indicating the presence of a slight or moderate degree of acidosis. The lowest figure of 7.10 was obtained in Case 5 when the patient was in a very poor condition with nausea and vomiting. The lowering of bicarbonate was, as a whole, more marked. Very rarely did it

exceed 20 milli-equivalents per liter, and the usual level was between 15 and 19. The chloride tended to be higher than normal in Cases 2 and 4. The phosphate showed some increase in most instances, but quantitatively the increase could only account for a small part of the loss of bicarbonate. The serum albumin was within the lower limits of normal in Cases 2 and 4, but definitely lower than normal in the rest, while the serum globulin was either normal or higher than normal. As a whole the sum of base-binding values of serum proteins was within normal limits. The sum of the anions fell short of the total base by a relatively wide margin. In Cases 3, 4 and 5, the undetermined anions varied from 6.5 to 13.2 milli-equivalents per liter. Sulphates may account for 1 or 2 m.-eq., but the rest are of unknown nature.

The total base was normal in Cases 3 and 4, but tended to be subnormal in Cases 2 and 5, especially the latter. In Case 5, the decrease of sodium was mainly responsible for the marked lowering of the total base. This patient was in poor condition with marked anorexia and vomiting during the latter part of the studies.

From the above observations, acidosis, as shown by the slight or moderate lowering of pH and marked decrease of bicarbonate, was present in all the cases. The decrease of bicarbonate was due not so much to an increase of phosphate as to an increase of undetermined anions. In Cases 2 and 4, the increase of chloride seemed to be a factor in the lowering of the bicarbonate. The presence of a chloride acidosis was found by Albright et al. (48) in a case of nephrocalcinosis with rickets and dwarfism and thought to be important in the causation of the bone disease. The loss of base contributing to the acidosis occurred only when intercurrent episodes of anorexia and vomiting, or terminal events had su ervened. It seems that in renal osteodystrophy, chronic acidosis is a constant feature and it is comparable in extent to that produced in experimental decalcification. However, in experimental acidosis in individuals with intact kidneys, an important phenomenon is the excessive urinary calcium loss, which is absent in renal osteodystrophy. In our cases the urine calcium remained small or absent. Therefore in renal osteodystrophy if acidosis has a decalcifying effect, the resorbed calcium is not drained through the kidneys. That it may be drained through the intestine is possible in view of the augmented calcium output in the stool in 2 cases of late rickets receiving ammonium chloride (30).

(b) *The effect of alkali and acid salt on calcium and phosphorus metabolism and serum electrolytes*

Experimental data of this type were available only from Case 2 (Fig. 3 and Table 5). The study was carried through 16 four-day periods with determinations of the serum electrolytes extended for some time before and after the metabolic observations. The diet contained 919 mg. calcium, 604 mg. phosphorus and 7.58 gm. nitrogen. Periods 21–24 served as control during which the positive calcium balance averaged 210 mg. per day and the positive phosphorus balance 214 mg. per day. The serum calcium tended to decrease from 5.11 to 4.57 mg.%, while the inorganic phosphorus rose from 6.25 to 7.54 mg.%. The

serum non-protein nitrogen came up from 127 to 168 mg.%. Acidosis increased in that the pH was 7.22 and bicarbonate was only 11.6 milli-equivalents per liter. The patient felt definitely worse so that she could not finish the diet in Period 24. During the next period the patient was given sodium bicarbonate, 80 cc. of molar solution (6.72 gm.) daily. This brought the serum pH up to 7.35 and bicarbonate up to 22.0 m.-eq. per liter. The serum N.P.N. went down to 131 mg.%, and continued to decrease during the subsequent periods. The serum inorganic phosphorus tended to decrease during the period of alkali administration, and the fall became more marked in the subsequent 3 periods when it reached 5.91 mg.%. Although the serum calcium level was somewhat depressed while the alkali was being given, it rose to its previous value immediately after

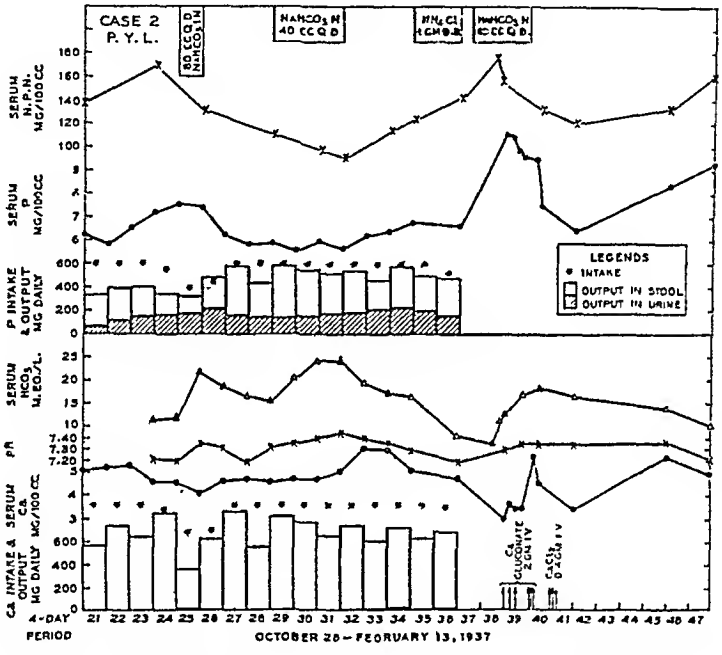

FIG. 3. CASE 2. INFLUENCE OF ALKALI AND ACID ON CALCIUM AND PHOSPHORUS METABOLISM AND ACID-BASE EQUILIBRIUM OF SERUM

the alkali was discontinued. While the food intake during the alkali period and the period after it was lower and irregular precluding conclusion as to the effect of the alkali on the calcium and phosphorus balance, the clinical improvement was so gratifying that further trial with the alkali was indicated.

By Period 27, the appetite of the patient had returned so that a quantitative consumption of the prescribed diet was again possible. After the control observations in Periods 27 and 28, sodium bicarbonate, 40 cc. of molar solution per day, was given in the 3 subsequent periods. In looking over the results on the calcium and phosphorus metabolism during Periods 29–33 (Fig. 3), one notices a progressive downward trend of the stool calcium giving rise to increasing calcium gain, as the alkali was being administered and for some periods after it was stopped. Although the average daily positive calcium balance of Periods 29–34 was not greater than that of the two preceding periods, the steady de-

crease of the stool calcium after alkali suggested that with the amelioration of the existing acidosis so that the pH and bicarbonate content approached normal (pH 7.40 and HCO_3 24.8 m.-eq. per liter), intestinal absorption of calcium improved, leading to better calcium balance. This improvement confirms the suggestion made before that acidosis may be associated with increased calcium loss in the stool. The increase of the serum calcium up to 6.09 mg.% was an additional evidence of improved calcium exchange. The phosphorus balance was not distinctly influenced by the alkali therapy except for a noticeable ascending trend in the urinary phosphorus elimination. The serum inorganic phosphorus was maintained below the 6 mg.% level during the alkali therapy, but it began to rise after the alkali was discontinued. Likewise after the N.P.N. decreased to a minimum of 91 mg.% at the end of alkali administration, it began to increase promptly.

Ammonium chloride in 1 gm. daily doses was given during Periods 35 and 36. The pH went down to 7.20 and bicarbonate to 8.3 milli-equivalents per liter. Thus the acidosis deepened considerably in spite of the small doses of the acid producing salt used. The serum N.P.N. rose to 142 mg.%. Clinically she felt worse again and could not finish the diet of the last period. The serum calcium and phosphorus were not much altered nor were the calcium and phosphorus balance significantly changed during the acid salt periods. However, the subsequent periods witnessed a dramatic rise of serum inorganic phosphorus and N.P.N., marked decrease of serum calcium and slight further depletion of serum bicarbonate. The patient had severe tetany with convulsions which were controlled by intravenous injections of calcium gluconate or chloride. The sodium bicarbonate solution given at this time might have aggravated the tetany, but it was responsible for correcting the acidosis and improving the renal function.

In this patient, although we were unable to witness a direct aggravating effect of ammonium chloride on the calcium and phosphorus balances, the deleterious influence on the renal function, the unfavorable changes in the serum levels of calcium and phosphorus and the systemic disturbance were impressive. The appetite was so much curtailed that adequate intake of all nutrients became impossible. Calcium intake would be especially short in view of the low calcium content of the Chinese diet. In this way chronic acidosis becomes a serious disturbing factor for the bone metabolism, not only by increasing the calcium loss through the intestine, but by curtailing the intake of all nutrients, especially calcium, through the appetite impairing influence of acidosis. The demonstration that the correction of the acidosis by alkali therapy led to an improvement of appetite and of calcium metabolism serves to emphasize the deleterious effect of chronic acidosis.

LACK OF RESPONSE TO VITAMIN D

Vitamin D, while specific in correcting the fundamental metabolic defects of rickets and osteomalacia and in rectifying the osseous changes, is without effect in cases of renal osteodystrophy. In fact, the lack of response to vitamin D has been taken as one of the important criteria for the diagnosis of the renal origin

of the bone disorder. Exceptional cases have been reported by György (49), Duken (50), and Karelitz and Kolomoyzeff (51) in which the improvement in the bone condition was attributed to vitamin D. But in view of the possible spontaneous remission of the renal insufficiency in certain types of kidney disease and the lack of metabolic data, it would be difficult to appraise the value of vitamin D in these cases.

In all the 5 cases of this series, vitamin D in the usual therapeutically effective doses was thoroughly tried, and the clinical and metabolic responses were uniformly absent. In two of the cases larger doses for longer periods were also tried without effect. In two other cases of the series, the single massive dosage (1,000,000 international units of vitamin D_2) was given. One of the patients exhibited an equivocal improvement in the calcium and phosphorus metabolism,

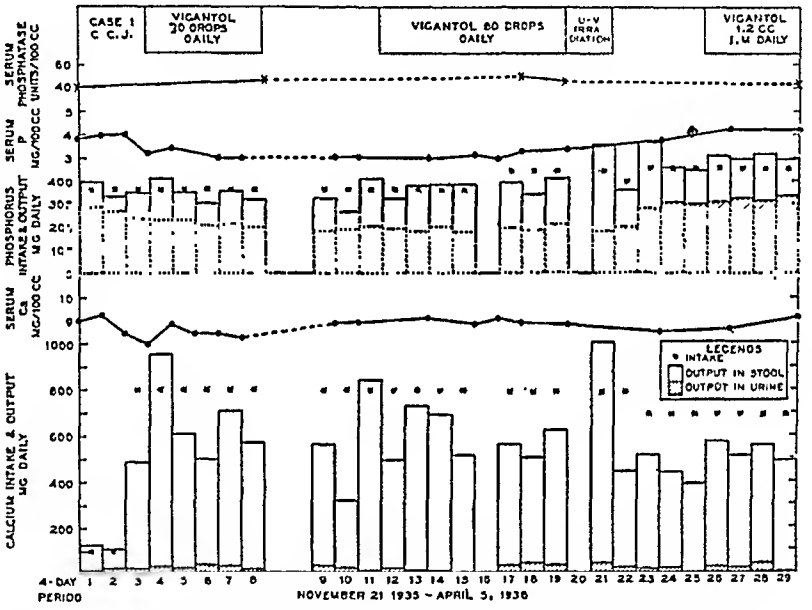

FIG. 4. CASE 1. EFFECT OF VITAMIN D ORALLY AND INTRAMUSCULARLY ON CALCIUM AND PHOSPHORUS METABOLISM

but the other patient's response was practically negative. In view of the different experimental set-up under which the observations on the vitamin D therapy were made in the various patients, it is necessary to comment on the results individually.

Case 1. After one control period (period 3) on a constant diet with 796 mg. of calcium and 358 mg. of phosphorus per day, Vigantol in 30-drop daily doses was given by mouth for 5 consecutive periods (Fig. 4). The daily calcium balance was 312 mg. during the control period and it averaged 131 mg. for the 5 therapy periods. The daily phosphorus balance, which was 13 mg. in the control period, averaged 10 mg. for the subsequent 5 periods. The serum calcium fluctuated irregularly between 8.03 and 8.85 mg.%, although the serum inorganic phosphorus decreased from 4.06 to 3.01 mg.% through the 6 periods of observation

After a lapse of 3 weeks during which tonsillectomy was performed, meta-

bolic studies were resumed. Periods 9–11 served as control during which an average of 334 mg. of calcium and 24 mg. of phosphorus was retained per day. Vigantol in 60-drop daily doses was given orally during Periods 12–19. This is equivalent to a total of 768,000 international units of vitamin D within 32 days. Ultraviolet irradiation was given during Periods 20–21. This massive dose of vitamin D, even with the addition of ultraviolet irradiation, did not bring about any improvement in the mineral metabolism. In fact, the calcium balance fluctuated irregularly and averaged 191 mg. per day for Periods 12–22; likewise the phosphorus balance was irregular, averaging 9 mg. per day for the same periods. The serum calcium remained relatively constant (8.82–9.12 mg.%), as well as the serum inorganic phosphorus (3.02–3.42 mg.%).

It was thought at the time that poor intestinal absorption might be responsible for the ineffectual oral dosage of vitamin D. Therefore, after 3 control periods (Periods 23–25) on a new diet containing 690 mg. calcium and 454 mg. phosphorus, Vigantol in 1.2 cc. daily doses was given intramuscularly for the next 5 periods. The intramuscular route of administration of vitamin D did not prove superior to the oral dosage. The daily calcium balance averaged 239 mg. in the control and 158 mg. during the intramuscular administration of vitamin D; and the daily phosphorus balance was 41 mg. before and −49 mg. during the therapy. There was but a slight increase of both serum calcium and inorganic phosphorus.

In contrast to ordinary rickets and osteomalacia in which the oral administration of 12,000 international units of vitamin D daily for 4 or 5 four-day periods was invariably followed by improved calcium and phosphorus absorption with large retention of these minerals (17, 52), this patient failed to respond to the same treatment. It has been demonstrated previously (53) that a patient with osteomalacia, having received vitamin D sometime previously and still showing good mineral balance, may respond only slightly or not at all to further dosage.

This was evidently not the case in this patient as she had had no prior vitamin D therapy in the history, and the relatively small extent of calcium retention and the practical absence of phosphorus retention during the first control period were not what one would expect in a patient who had responded to previous vitamin D therapy. Therefore one has to conclude that this patient was incapable of responding to vitamin D therapy. The subsequent results with the second series with rather massive dosage by mouth and the third series by the intramuscular route add to the strength of this conclusion.

Case 2. Vigantol 1 cc. daily was given for 20 days (Periods 10–14), while the patient was on a diet with 1207 mg. calcium and 675 mg. phosphorus per day (Fig. 1). The average daily calcium balance was 173 mg. and the average phosphorus balance 8 mg. As the vitamin D periods followed the administration of sodium phosphate (Periods 8–9) which accounted for the negative phosphorus balance during the subsequent two periods, only Periods 12–14 should be taken in computing the average phosphorus balance which then came up to 61 mg. per day. These figures represent very slight, if any, improvement over those during Periods 6–7 which may be taken as control, namely 63 mg. of calcium and −56 mg. of phosphorus per day. Likewise the changes in the serum

calcium (from 5.91 to 6.54 mg.%) and inorganic phosphorus (from 6.29 to 5.96 mg.%) were of doubtful significance.

Case 3. After a control observation for 5 periods (Periods 1–5) on a calcium intake varying from 620 to 856 mg. and a phosphorus intake from 486 to 613 mg. per day, during which the daily calcium balance averaged 105 mg. and phosphorus balance 73 mg., Vigantol 1 cc. daily was given for 5 periods and 5 cc. daily for the next 2 periods. Thus a total of 720,000 international units of vitamin D was administered within 28 days. This constitutes a massive dose. Metabolic observations (Fig. 5) were made only during the last 4 periods (Periods 10–13). The calcium balance averaged 63 mg. and phosphorus balance −9 mg. per day.

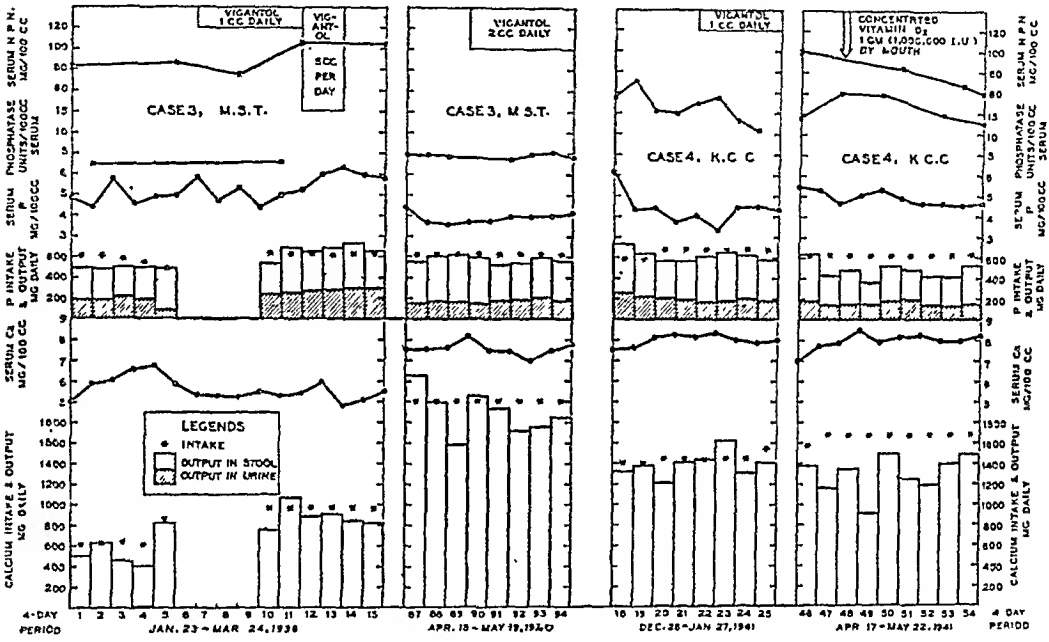

FIG. 5. CASES 3 AND 4. EFFECT OF VITAMIN D OF VARIOUS DOSAGE ON CALCIUM AND PHOSPHORUS METABOLISM

Thus the daily balances were even less favorable than during the control periods. This is all the more significant when it is noted that both the calcium and phosphorus intake were higher during the vitamin D periods than during the control. The serum calcium and inorganic phosphorus remained practically constant throughout.

Vigantol was given again a year later in 2 cc. daily doses for 5 periods (Periods 90–94) on a diet with 2009 mg. calcium and 626 mg. phosphorus. The daily calcium balance on this regimen averaged 146 mg. and phosphorus balance averaged 65 mg. These were not significantly different from those of the 3 preceding control periods. Nor were there clearcut changes in the serum calcium and inorganic phosphorus (Fig. 5).

Case 4. In this patient vitamin D was tried both in ordinary therapeutic doses and in a single massive dose (Fig. 5). Control observations were made

during Periods 20 and 21 on a diet with 1451 mg. calcium and 678 mg. phosphorus per day. The patient retained on the average 136 mg. calcium and 113 mg. phosphorus daily, with the serum calcium slightly above 8 mg.% and inorganic phosphorus slightly above 4 mg.%. Vigantol 1 cc. daily was exhibited for Periods 22–25 inclusive, during which the calcium balance averaged 29 mg. and phosphorus balance averaged 60 mg. per day. The serum calcium and inorganic phosphorus showed no significant change. The observations for the next 3 periods, in which the vitamin D administration had been discontinued, likewise showed no substantial improvement in the calcium and phosphorus metabolism (See Fig. 7, Periods 26–28).

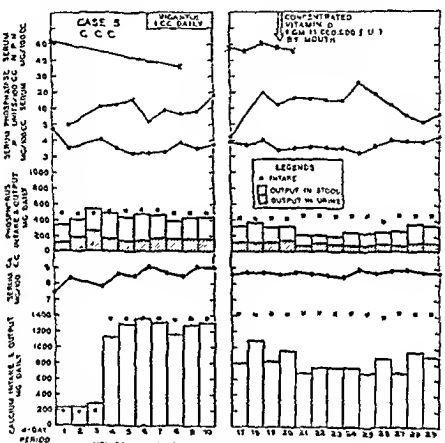

FIG. 6. CASE 5. EFFECT OF VITAMIN D OF VARIOUS DOSAGE ON CALCIUM AND PHOSPHORUS METABOLISM

On the first day of Period 48 the patient received by mouth 1 gm. of a concentrated solution of irradiated ergosterol containing 1,000,000 international units of vitamin D. The calcium intake was high (1572–1677 mg.) and phosphorus intake relatively low (624 mg.). During the control (Periods 46–47) the calcium retention was 358 mg. and phosphorus retention was 88 mg. per day; while during the next 7 periods (Periods 48–54) following the ingestion of the massive dose of vitamin D, these figures were respectively 382 and 163 mg. The serum calcium (7.91–8.15 mg.%) and inorganic phosphorus (4.63–4.60 mg.%) remained essentially constant. The response, then, even to a massive dose of vitamin D was negligible.

Case 5. This patient like the preceding one, received vitamin D at two dosage levels (Fig. 6). During Periods 7–10, Vignatol in 1 cc. daily doses was given. The average calcium balance was 96 mg. and phosphorus balance was 66 mg.

per day. These figures were not different from those of the control periods (Periods 4–6), which were 96 and 53 mg. respectively. The serum calcium remained at approximately 9.0 mg.%, while the inorganic phosphorus increased slightly from 3.26 to 3.74 mg.%. Thus the response to ordinary therapeutic doses of vitamin D was practically negative.

The single massive dose of vitamin D (1,000,000 i.u.) was given orally on the first day of Period 20. The observations of the 3 preceding periods (Periods 17–19) served as control in which the retention of calcium amounted to 515 and that of phosphorus to 79 mg. per day. Following the massive dose of vitamin D, the average calcium balance for the subsequent 10 periods (Periods 20–29)

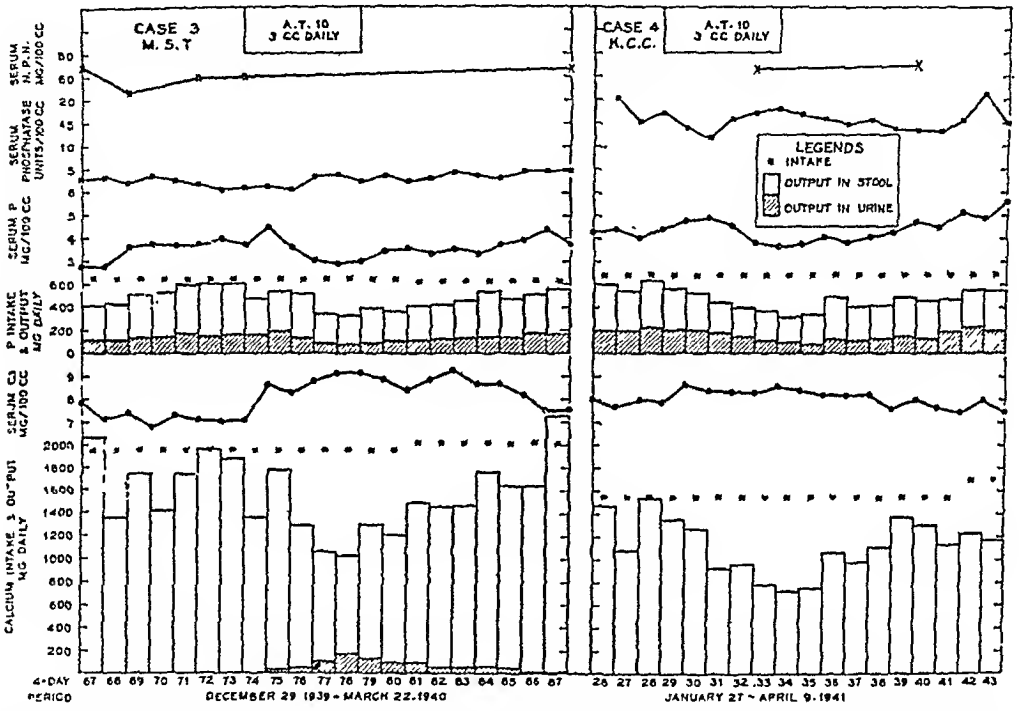

FIG. 7. CASES 3 AND 4. INFLUENCE OF DIHYDROTACHYSTEROL (A.T.10) ON CALCIUM AND PHOSPHORUS METABOLISM

was 629 and the corresponding phosphorus balance was 193 mg. per day. While both the calcium and phosphorus balances were somewhat improved, the improvement was slight or equivocal in comparison with the remarkable results on osteomalacia from similar dosage (54). The serum calcium remained constant, being 8.53 mg.% on the day of the massive dose and 8.61 mg.% at the end of the 10 periods. The serum inorganic phosphorus rose during the same period of time from 3.33 to 4.18 mg.%.

Summary. All the patients were given ordinary therapeutic doses of vitamin D and none showed any improvement in the calcium and phosphorus metabolism. Two of the patients (Cases 1 and 3) received also much larger dosage for longer periods (respectively 768,000 and 720,000 i.u. in toto) likewise without appreciable improvement. Of the two patients receiving the single massive

dose (1,000,000 i.u.), one (Case 4) showed practically no response and the other (Case 5) a very slight response from the standpoint of mineral metabolism. Vitamin D in ordinary therapeutic doses was given intramuscularly in Case 1, and no favorable response was elicited.

The lack of response to vitamin D may be taken to mean that renal osteodystrophy bears no etiological relationship to vitamin D. If so, one would not expect improvement in renal osteodystrophy from vitamin D therapy any more than hyperparathyroidism or osteogenesis imperfecta would be improved by the same treatment. However the anatomical changes in the bones associated with renal insufficiency may at times be indistinguishable from rickets and osteomalacia (55, 56). And both types of bone disease have certain metabolic defects in common, namely poor absorption of calcium through the intestine with large quantities of calcium in the stool, absence or negligible amounts of calcium in the urine, and hypocalcemia. Thus there is still room for the consideration that renal osteodystrophy is related to vitamin D not from any deficiency in the intake of the vitamin or in the exposure to sunlight as in osteomalacia and rickets, but from an interference with the action of the vitamin conditioned by renal insufficiency. With advanced renal impairment phosphate is retained in the blood and tissue fluids, leading to a high concentration in the intestine. The presence of large amounts of phosphate in the bowel precipitates calcium, rendering the latter difficult of absorption. While vitamin D improves the absorption of calcium in conditions where poor absorption is due to vitamin D deficiency, it may not be able to remedy a similar difficulty arising from the calcium being diverted to enable phosphorus elimination by the intestine. Such a viewpoint may hold true in advanced renal insufficiency such as Cases 2–5, where high concentration of inorganic phosphorus in the serum, large stool phosphorus and small urinary phosphorus excretion did exist. But in Case 1 where the renal impairment was only moderately severe, and consequently the urinary tract still constituted the main route of phosphorus elimination, and the serum inorganic phosphorus remained at normal level, the ineffectiveness of vitamin D in promoting calcium absorption could not be due to an interference by the high stream of phosphorus going from the blood to the intestine. We have seen cases of osteomalacia with only a slight impairment of renal function, but already showing poorer, slower or less well-sustained improvement in mineral metabolism after vitamin D therapy (53, Case 1). An alternative explanation which will take into consideration those milder cases of renal damage showing poor or no response to vitamin D would be that in renal insufficiency a certain factor is produced or retained which inactivates vitamin D, rendering it ineffectual. Such a hypothesis is difficult to investigate. But as shown in the following section, A.T.10 is effective in promoting calcium and phosphorus absorption and deposition in the bones in renal osteodystrophy just as in ordinary osteomalacia. This lends support to the hypothesis of vitamin D inactivation, and further suggests that such an inactivation, if it exists, is chemically specific for vitamin D and not generally applicable to other calcium absorption promoting compounds like A.T.10.

FAVORABLE EFFECT OF DIHYDROTACHYSTEROL (A.T.10)

Dihydrotachysterol (A.T.10) is one of the products of irradiation of ergosterol, first used by Holtz in 1933–34 (57, 58) for the treatment of hypoparathyroid tetany with prompt rise of serum calcium and relief of symptoms. The efficacy of A.T.10 in the therapy of various forms of tetany has been confirmed by a host of observers (59–65). Metabolic observations by Albright and co-workers (66) on hypoparathyroidism showed that A.T.10 promotes calcium absorption by the intestine and increases phosphorus elimination by the kidneys. In rickets they (67) observed that while calcium is absorbed and retained after A.T.10 therapy, very little phosphorus is retained, thus concluding that this compound is not antirachitic. However Shohl and co-workers (68, 69) demonstrated antiricketic effect of A.T.10 in experimental rickets in the rat. Our own observations on 3 cases of osteomalacia (70) show that A.T.10, like vitamin D, promotes calcium absorption by the intestine followed by increased phosphorus absorption. The absorbed elements are retained for the replenishment of the depleted bone store and for the elevation of the serum calcium and phosphorus levels when these are low. While these favorable effects on osteomalacia from A.T.10 wear off quickly after its discontinuation in contrast to the prolonged after-effect of vitamin D, they are striking while the therapy is being given. It is therefore of interest and importance to ascertain the effect of A.T.10 in renal osteodystrophy where the difficulty of intestinal absorption cannot be rectified by vitamin D administration. Metabolic observations on A.T.10 are available in 2 cases of renal osteodystrophy in this series.

Case 3. The control observations were made during Periods 67–73 on a constant diet with 1952 mg. of calcium and 642 mg. of phosphorus per day (Fig. 7). The calcium output was all in the stool, and the average daily positive balance was 217 mg. The phosphorus excretion was 27% in the urine and the daily average positive balance was 170 mg. Throughout the 7 control periods the serum calcium fell from 7.87 to 7.08 mg.%, while the serum inorganic phosphorus rose from 2.77 to 3.72 mg.%. These changes in the serum levels of calcium and phosphorus were due to the discontinuation of ferric ammonium citrate therapy (see Section on Effect of Iron and Fig. 8). A.T.10 was given by mouth in 3 cc. daily doses for 5 periods (Periods 74–78). One notices immediately a decrease of both stool calcium and phosphorus with improved balances. As the therapy was continued, calcium began to appear in the urine in significant amount and by the 5th period of the treatment it amounted to 163 mg. per day—a remarkable figure considering the fact that in this as well as in other patients with renal osteodystrophy, absence or presence of a trace only of urinary calcium was a constant finding. The stool calcium continued to diminish so that during the last period of A.T.10 treatment, in spite of the large urinary calcium, the daily balance amounted to 927 mg. or 47% of the intake. The average daily calcium balance for the 5 periods together was 652 mg. or 33% of the intake. The phosphorus excretion in the stool, as well as in the urine, also steadily diminished so that the daily phosphorus retention during the last A.T.10 period came up to 318 mg. or 50% of the intake. The average daily phosphorous balance was

207 mg. or 33% of the intake. The serum calcium rose from 7.08 to 9.18 mg.% through the 5 periods of A.T.10 administration, while the serum inorganic phosphorus showed a diminution from 3.72 to 3.04 mg.%.

After A.T.10 therapy was discontinued from Period 79 the stool calcium began to increase and urinary calcium began to decrease so that in the course of 8 or 9 periods the pre-A.T.10 state was reached. Likewise the stool phosphorus, as well as the urinary phosphorus gradually increased to approach the previous values in the same period of time. The serum calcium decreased from 9.18 to 7.52 mg.% and phosphorus increased from 3.04 to 4.39 mg.% at the end of Period 86.

Case 4. Periods 26–28 served as control with a calcium intake of 1550 mg. and a phosphorus intake of 678 mg. per day (Fig. 7). The output of calcium was all in the stool leaving an average daily balance of 153 mg.; while the phosphorus elimination was 34% in the urine with an average daily balance of 86 mg. The serum calcium remained at 8 mg.% and the serum inorganic phosphorus at a little over 4 mg.%. A.T.10 was given by mouth during Periods 29–33 in 3 cc. daily doses. Like the preceding case, both the calcium and the phosphorus in the stool began to decrease during the first therapy period and continued to to do so throughout the following periods. Unlike the previous patient the urinary calcium remained absent or in negligible amount, although the urinary phosphorus diminished steadily. The maximum retention of calcium was 815 mg. and that of phosphorus was 359 mg. per day; both amounted to 53% of their respective intake and occurred during Period 34, the period just after the discontinuation of the A.T.10 administration. The serum calcium rose from 7.92 to 8.55 mg.% and the serum inorganic phosphorus fell from 4.41 to 3.68 mg.%.

The post-control observations showed a gradual reversal of the above described changes. From Period 35 onward, the stool calcium and phosphorus, as well as the urinary phosphorus, steadily increased so that the calcium balance approached the pre-A.T.10 level in Period 39, the 6th period after the withdrawal of the therapy, and the phosphorus balance behaved similarly 3 or 4 periods later. The serum calcium decreased from 8.55 to 7.40 mg.% and the serum inorganic phosphorus rose from 3.68 to 5.14 mg.% in the course of 8 periods (from Period 34 to 41).

Summary. The favorable influence of dihydrotachysterol on the calcium and phosphorus metabolism in renal osteodystrophy is remarkable. The primary action of this drug seems to be the improvement of the intestinal absorption of calcium and also of phosphorus, thus reducing both the stool calcium and phosphorus. A small part of the absorbed calcium was eliminated by the kidneys in Case 3 and none appeared in the urine in Case 4. The maximal retained calcium amounted to 47% and 53% of the intake respectively. The conservation of phosphorus, on the other hand, was brought about by a diminution of both stool and urine elimination. The maximum daily phosphorus retention recorded for the two patients was respectively 50 and 53% of the intake. While a minute part of the retained calcium went to increase the serum level, the major

portion must be deposited, together with the retained phosphorus, in the tissues, presumably the bones. The phosphorus deposited in the tissues is contributed to also by that present in the serum, as there was a fall in the level of serum inorganic phosphorus in both cases during the therapy.

The above described metabolic results in renal osteodystrophy from A.T.10 therapy are somewhat superior to those in ordinary osteomalacia from the same treatment (70) and the favorable after-effects of the therapy appear to last somewhat longer in the former than in the latter condition. Compared with the results of the specific vitamin D therapy in rickets and osteomalacia, the maximum mineral retention brought about by A.T.10 in renal osteodystrophy is just as excellent. In fact the improvement of calcium and phosphorus metabolism in renal osteodystrophy with the administration of A.T.10 appeared so encouraging that we venture to suggest that if it were possible to maintain the therapy for a sufficiently long period of time, the skeletal decalcification would be rectified and the general well being of the patient would be improved.

That dihydrotachysterol promotes absorption and retention of calcium and phosphorus in osteomalacia with normal kidneys as well as in renal osteodystrophy indicates that renal insufficiency makes no difference to its action. In contrast to A.T.10, vitamin D is incapable of exerting similar effect as soon as renal insufficiency supervenes. The fact that this is true of milder grades of renal functional impairment without retention of phosphate and shift of phosphate excretion from the kidneys to the intestine suggests that it is not so much the peculiar type of difficulty of intestinal absorption in renal insufficiency that can not be surmounted by vitamin D as a nullification of its action inherent in renal insufficiency. Specific inactivation of vitamin D as the result of a factor retained or produced in renal functional impairment is a distinct possibility, although no direct proof is available.

EFFECT OF IRON

In the course of a study of a case of osteomalacia in pregnancy the coexisting anemia indicated the use of iron. Large doses of ferric ammonium citrate were then given. While the anemia did not improve, it was accidentally discovered that the serum inorganic phosphorus decreased and calcium slightly increased, associated with a marked increase of the stool phosphorus and a slight decrease of the phosphorus balance (71). In view of the well known fact in elementary chemistry that a soluble ferric salt precipitates phosphate from a solution by forming insoluble ferric phosphate, similar chemical reaction probably happens in the intestinal canal. Such a precipitation would limit the absorption of phosphorus and thus lower the serum inorganic phosphorus. The serum calcium would rise partly in response to the fall of the inorganic phosphorus and partly due to the calcium-sparing action of iron in precipitating phosphate in the intestinal tract. Such a procedure should remedy the situation encountered in renal osteodystrophy where the phosphate retention leads to a high concentration of phosphate in the intestine which, in turn, precipitates calcium and limits its absorption. Ferric ammonium citrate was accordingly tried on two patients of this series, and the metabolic observations are as follows:

Case 3. On a constant diet with 1978 mg. of calcium and 707 mg. of phosphorus, the control observations during Periods 30–32 showed that the average daily retention of calcium was only 67 mg. and that of phosphorus −22 mg. (Fig. 8). The serum calcium tended to increase (from 5.14 to 5.82 mg.%) and inorganic phosphorus to lower (from 4.19 to 3.72 mg.%). Ferric ammonium citrate 6 gm. daily was started from Period 33 and continued through Period 45 (altogether 52 days) after which the patient was discharged (July, 1939). The metabolic observations were made only during the first two periods of iron therapy in which the stool calcium was definitely decreased giving rise to an average daily gain of 410 mg. of calcium, and the phosphorus balance also showed slight improvement, averaging 32 mg. per day. The serum calcium and inorganic phosphorus were followed up throughout the periods of iron therapy when

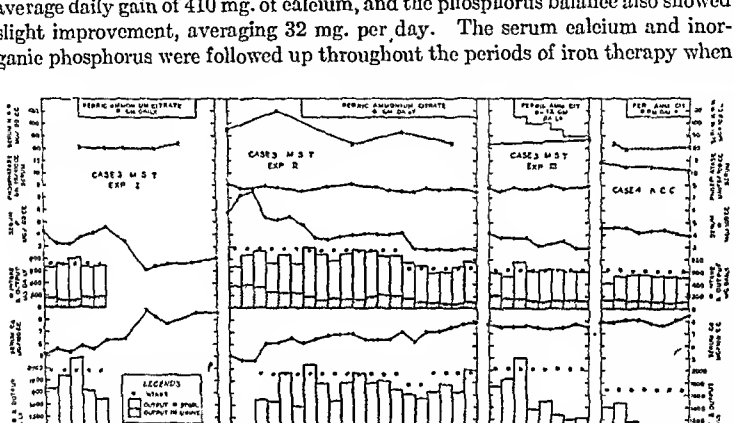

FIG. 8. CASES 3 AND 4. EFFECT OF IRON ON CALCIUM AND PHOSPHORUS METABOLISM

the high calcium intake was also maintained. The serum calcium gradually rose from 5.82 to a maximum of 9.00 mg.% during Period 38; from then on it fell somewhat but maintained a little over 8 mg.%. The serum inorganic phosphorus level initially tended to rise from 3.72 to 4.60 mg.%, but later it fell rather precipitously to a minimal value of 1 mg.% during Period 38. After this minimum had been reached, it gradually increased to and maintained itself at 2 mg.%.

The patient was readmitted in October 1939 and metabolic observations were repeated. From Period 49 to 52 the control observations on a diet with 1915 mg. of calcium and 962 mg. of phosphorus revealed an average daily balance of 346 mg. of calcium and 146 mg. of phosphorus (Fig. 8, Exp. II). The serum calcium rose from 4.74 to 6.14 mg.% and the serum inorganic phosphorus fell from 7.60 to 4.90 mg.%, apparently from an increase of the calcium intake as discussed under the Section on Importance of Calcium Intake. But the figures

for the last of the 4 control periods may be taken as the base line for subsequent comparison. Ferric ammonium citrate 6 gm. daily was given from Period 53 to 66. During these 14 periods of iron therapy the calcium balance fluctuated irregularly but on the average it was less than during the control periods, namely, 157 mg. per day. The phosphorus balance was also less than the control, being 39 mg. per day for Periods 53–60, and −41 mg. per day for Periods 61–66 when a lower phosphorus containing diet was used. The serum calcium increased from 6.14 to 7.87 mg.% in the course of the 14 periods of iron therapy, while the serum inorganic phosphorus decreased from 4.90 to 2.77 mg.%, the rate of decrease being accelerated during the last 6 periods of lower phosphorus intake. These changes could be properly attributed to the iron administration because during the next 4 periods (Periods 67–70, Fig. 7) without iron, both the calcium and phosphorus balances increased to 310 and 170 mg. per day respectively with a fall of the serum calcium and a rise of the serum inorganic phosphorus.

In April-May 1940, the patient was observed for the third time in regard to the effect of iron therapy (Fig. 8, Exp. III). The control periods (Periods 95–96) followed the cessation of Vigantol administration which apparently had no effect on the mineral metabolism. During these periods the average daily retention consisted of 216 mg. of calcium and 76 mg. of phosphorus. Ferric ammonium citrate was then administered in the following daily doses: 6 gm. in Period 97, 8 gm. in Periods 98–99, 10 gm. in Period 100 and 12 gm. in Periods 101–102. The patient tolerated well the gradual increase of the dosage. The calcium balance showed marked improvement, averaging 564 mg. daily, while the phosphorus balance a slight but definite decrease, amounting to −16 mg. per day. The serum calcium, however, remained almost unchanged at the level of 7.50 mg.%, while the serum inorganic phosphorus decreased from 3.70 to 2.96 mg.%.

Case 4. This patient received iron towards the end of a long series of metabolic studies. The control observations during Periods 53–54 showed an average daily retention of 238 mg. of calcium and 149 mg. of phosphorus. The serum calcium (7.91–8.15 mg.%) and inorganic phosphorus (4.55–4.60 mg.%) were fairly constant. Ferric ammonium citrate in 6 mg. daily doses was given from Period 55 to 59, during which the calcium balance improved markedly, averaging 604 mg. per day, while the phosphorus showed a slight but definite decline, the average being 74 mg. daily. The serum calcium rose slightly from 8.15 to 8.43 mg.%, while the serum inorganic phosphorus fell from 4.60 to 3.94 mg.% through the periods of iron administration (Fig. 8).

Summary. The data on the administration of iron in the two cases are summarized in Table 6. The results are on the whole favorable. The most consistent change was a decline of the serum inorganic phosphorus level. The decline was definite in all the four trials, and most pronounced in the first trial in Case 3. As a rule the serum calcium tended to rise. This tendency was marked in the first and second trials in Case 3, slight in Case 4 and absent in the third trial in Case 3. It is to be noted that in the latter two instances the initial values of 7.50 and 8.15 mg.% were much higher than those in the former, sug-

gesting that the initial height of the serum calcium level bore an inverse relationship to the extent of improvement attainable through the administration of iron.

The phosphorus balance showed a decline in all instances except the first trial in Case 3 in which the increase of phosphorus retention was relatively slight and the results obtained were from only two metabolic periods. When a decline in phosphorus balance occurred, it was invariably brought about by an increase in the stool excretion of phosphorus, thus compatible with the hypothesis that iron precipitates phosphate in the intestine and limits its absorption.

In the second trial in Case 3, the calcium balance decreased slightly following iron therapy, but it registered a substantial increase in all the rest. It was difficult to explain the decrease of calcium balance in that particular instance except that iron sometimes gives diarrhea and calcium may not be absorbed because of hurried bowel motility. Although there was no actual diarrhea during the second trial of iron therapy, the stool weight and nitrogen were higher than in the rest, suggesting increased intestinal activity. However the majority of instances were associated with an improvement in the calcium balance and the improvement was considerable. This indicates that iron does, as a rule, increase calcium absorption probably through its calcium-sparing action in combining with phosphate in the intestine.

The influence of iron on the calcium and phosphorus metabolism has received considerable attention experimentally. Cox, Dodds, Wigman and Murphy (72) demonstrated that aluminum or ferric iron added to the ration of guinea pigs and rabbits caused marked lowering of the bone ash and serum phosphorus. Using rats and adding ferric chloride to a non-rachitogenic ration, Brock and Diamond (73) produced severe rickets. Deobald and Elvehjem (74) found that chicks placed on a normal ration to which was added large amounts of soluble iron or aluminum salts developed severe rickets in 1 or 2 weeks. These authors warned against the possible danger of using large doses of iron in the treatment of hypochromic anemia. Rhem and Winters (75) found that the addition of ferric chloride to combine with one-half of the phosphorus of the diet of the rat resulted in a considerable reduction in the ash, calcium and phosphorus contents of the bodies of the animals. The influence of high phosphorus on iron metabolism has also been studied (76–78).

From the standpoint of human nutrition, Barer and Fowler (79) found that in a series of 19 patients with anemia or arthritis, the administration of ferric ammonium citrate up to 3 gm. per day failed to give rise to significant or consistent alteration in the calcium, phosphorus or nitrogen balance. Iron therapy in renal osteodystrophy with the view to facilitating the excretion of phosphate by the intestine and thereby to lower the serum phosphate has not been reported in the literature, as far as we are aware. The fact that such results could be accomplished in two cases reported above justifies further trial with this form of medication in other cases of renal osteodystrophy. The calcium balance is usually improved, but at times adversely affected by the ingestion of large doses of ferric salts in instances where diarrhea or increased bowel activity is induced. The presence of anemia in renal osteodystrophy seems to be an additional in-

dication for the iron therapy, but this is doubtful because the anemia usually does not respond to such treatment.

PATHOGENESIS

The foregoing observations demonstrate that in advanced renal insufficiency the excretion of phosphorus by the kidneys is hampered, resulting in an elevation of serum phosphorus and appearance in the stool of larger amounts of phosphorus than normal under similar levels and ratios of calcium to phosphorus intake. The increased stool phosphorus elimination is accompanied by a negative calcium balance when the dietary calcium is low or moderate and by a relatively small calcium retention when the calcium intake is high. Thus the necessity of intestinal excretion of phosphorus hinders calcium absorption, supporting the theory of Mitchell that a shift of phosphorus excretion from the kidneys to the intestine results in calcium starvation and thereby osseous disorder. A more striking demonstration of the same train of events is afforded by increasing the phosphorus intake. Under increased strain, the serum phosphorus shows further elevation and the intestinal excretion of phosphorus assumes greater prominence. The excessive intestinal excretion of phosphorus tends to last for some time after the increased phosphorus feeding is discontinued. The calcium loss becomes evident during these post-phosphorus feeding periods when negative phosphorus balance prevails on account of the excess phosphorus being slowly eliminated by the intestine.

While the excessive intestinal excretion of phosphorus by reason of renal insufficiency plays an important role in the genesis of the osseous disorder, we believe that chronic acidosis usually present in such cases is also a serious factor. It is true that when acidosis induced in individuals with normal kidneys drains the skeletal store, it is evidenced by an excessive calcium and phosphorus output in the urine; and that this is absent in renal osteodystrophy. But in the latter condition, failing excretion by the kidneys, the minerals resorbed from the bones may be disposed of through other channels. The possibility that part of the excess calcium and phosphorus present in the intestine is derived from the skeleton on account of the decalcifying effects of acidosis cannot be excluded. Apart from its possible direct effect on the skeleton, acidosis aggravates the general condition of the patient, leading to impairment of appetite, thus curtailing the intake of all nutrients, including calcium. Chronic calcium starvation would be of serious import to the bone metabolism.

This leads to the consideration of a third factor in the genesis of the skeletal disorder in renal osteodystrophy which is especially important in China. The prevailing low calcium intake in the usual Chinese dietary is incapable of maintaining patients with advanced renal insufficiency in calcium balance. These patients require greater amount of calcium than normal in order to offset the augmented intestinal output of calcium. Therefore subsistence on a low calcium diet would render the osseous disease manifest earlier or more acutely than on a high calcium intake when renal insufficiency exists.

All the three factors enumerated above contribute to produce a lack or deficiency in the absorption or assimilation of calcium, which must have an impor-

tant bearing on the skeletal decalcification in renal osteodystrophy. Such a defective mechanism of absorption and assimilation is not susceptible to rectification by the administration of vitamin D which is specific in remedying a similar defect in rickets and osteomalacia. One might suppose that the defective absorptive state in renal insufficiency, being brought about by the necessity of large intestinal excretion of phosphorus, differs fundamentally from that in rickets or osteomalacia, and is therefore not expected to improve by vitamin D. This supposition may hold true in advanced renal insufficiency in which phosphate retention and large phosphate excretion by the intestine have been demonstrated to be present, but it can not be applied to cases of milder renal functional impairment where such phenomena are not yet evident. The fact that milder cases of renal insufficiency with associated osteodystrophy are also incapable of being improved by vitamin D speaks for some alternative hypothesis to explain its inefficacy. It is possible that renal functional impairment even before it is advanced results in a condition by which vitamin D is inactivated or otherwise nullified in its action of promoting calcium absorption. The fact that A.T.10 promotes calcium absorption equally as well in renal osteodystrophy as in osteomalacia and rickets suggests that the difficulty in calcium absorption in the former condition can be surmounted. The efficacy of A.T.10 in this respect cannot be attributed to its stronger action in promoting calcium absorption, compared with vitamin D, for this is not found to be the case in comparative studies of the two drugs in hypoparathyroidism (66) and in osteomalacia (70). It seems as if A.T.10 is able to exert its full effect irrespective of the status of renal function, while vitamin D is hampered in its action as soon as renal function is interfered with. In other words, there seems to be a specific inactivation of vitamin D conditioned by renal insufficiency.

If the above hypothesis is correct, renal osteodystrophy may be regarded as a result of vitamin D deficiency, not from a lack of its intake, but from its inactivation peculiar to renal insufficiency. In this sense, terms like renal rickets and renal osteomalacia may find their full justification. The clinical and pathological picture of these conditions need not be identical with that of rickets and osteomalacia in view of such modifying factors as chronic acidosis and hyperparathyroidism secondary to renal disease.

The data on the present series of cases do not help to decide whether secondary hyperparathyroidism is an important factor in the causation of the bone disease in renal osteodystrophy. While this hypothesis has been advocated in view of the frequent occurrence of diffuse parathyroid hyperplasia and of osteitis fibrosa cystica in cases of renal osteodystrophy, it has not been generally accepted. The presence of rickets-like changes in the epiphysis which are absent in primary hyperparathyroidism in children, as pointed out by Albright et al. (2), is a serious objection, among others, to the theory of secondary hyperparathyroidism being responsible for the bone changes in renal osteodystrophy.

TREATMENT

The ultimate prognosis in renal osteodystrophy is bad in view of the irreparable kidney damage in these cases, but we feel that certain procedures may be

instituted to improve the general condition of the patient, to alleviate the acidosis and to promote calcium and phosphorus assimilation. In this way the general well being of the patient will be enchanced and the bone condition will be ameliorated.

1. Diet

In view of the renal functional impairment, a diet, limited in protein and phosphorus contents but optimal in all other respects, should be given. The daily intake of phosphorus should not exceed 500–600 mg. A large intake of calcium is also necessary to offset the drain on the skeletal store. A minimum of 1 gm. of calcium a day should be taken. If this intake can not be attained by dietary means, it should be supplemented by calcium lactate or calcium gluconate. The improvement reported by Salvesen (47) in his case of renal rickets was attributed to a large calcium intake.

2. Alkali

In view of the deleterious effects of acidosis, this should be corrected. The administration of sodium bicarbonate or a sodium citrate—citric acid mixture as used by Albright et al. (48) in a case of nephrocalcinosis is indicated. The dosage is so adjusted as to maintain a normal pH and bicarbonate content of the serum. Schoenthal and Burpee (35) and Graham and Oakley (80) have stressed the value of alkali administration in renal rickets. They find that correction of acidosis results in improvement in renal function. With this our observations agree; and in addition a normal acid-base status seems to favor calcium assimilation. The only drawback of alkali administration is the tendency to produce tetany. In such cases either the dosage of alkali must be reduced or intravenous calcium gluconate be given.

3. Dihydrotachysterol

Since one of the fundamental defects of renal osteodystrophy is poor intestinal absorption of calcium, therapy should be directed to the circumvention of this difficulty. Vitamin D is ineffective in this regard. Dihydrotachysterol (A.T.10) was found to promote calcium absorption in 2 of our cases in which this treatment was tried. The increased calcium absorption led to increased phosphorus absorption and to deposition in the bones of both of these elements. This form of therapy appears to be so gratifying that it should be tried in other cases. In view of its after-effect being not long sustained following its discontinuation, A.T.10 ingestion should be maintained for prolonged periods in order to secure substantial replenishment of the skeletal store.

4. Iron

Ferric ammonium citrate facilitates the intestinal excretion of phosphorus by the formation of insoluble ferric phosphate. This spares the amount of calcium necessary for combining with phosphate to enable the excretion of the latter by the intestine. Hyperphophatemia, an evidence of strain on the kidneys, is usually promptly relieved by the ingestion of iron. Therefore such therapy

is indicated in cases of renal osteodystrophy with marked elevation of serum inorganic phosphorus. Occasionally iron, in large doses, causes diarrhea, and in such cases it should be discontinued.

SUMMARY AND CONCLUSIONS

1. Metabolic observations on 5 cases of renal osteodystrophy are presented. The bone lesions varied from slight or moderate osteoporosis with no deformities to marked rarefaction with gross deformities. In two cases rachitis-like changes were also present in the epiphyses. The nature of the kidney disease in these cases was not determined; the renal insufficiency was moderate in one case and far advanced in the rest.

2. In the cases of advanced renal insufficiency difficulty in renal excretion of phosphate was evidenced by a relatively small percentage of urinary phosphorus, and by hyperphosphatemia. Such evidence could be brought out more clearly by increasing the phosphorus intake. Calcium balance was negative on low or moderate calcium intake and slightly positive on high calcium intake. Large stool excretion of phosphorus after increased phosphorus intake was usually followed by calcium loss even on high calcium intake.

3. Chronic acidosis of moderate degree was present in all the cases. Alkali administration alleviated the acidosis with improvement of renal function and favorable effect on the calcium metabolism.

4. The response to ordinary therapeutic doses of vitamin D was absent in all the cases, and it was meager even to massive doses. In contrast to vitamin D, dihydrotachysterol (A.T.10) was found to be remarkably effective in promoting calcium and phosphorus absorption and their deposition in the skeletal store. Iron was found to be efficacious in relieving hyperphosphatemia and in sparing calcium in the intestinal elimination of phosphorus.

5. In the pathogenesis of renal osteodystrophy the lack of response to vitamin D even in cases of mild degree of renal insufficiency was thought to be significant and to suggest the possibility of inactivation of vitamin D as a result of renal functional impairment. This was considered to be of primary importance in the genesis of the bone disease. A shift of phosphate excretion from the kidneys to the intestine and marked acidosis, only present in advanced renal insufficiency, were important aggravating factors.

6. For treatment, a low phosphorus, low protein and high calcium diet, alkali, dihydrotachysterol and iron were indicated. The results with dihydrotachysterol were particularly recommendable.

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APPENDIX

Case 1. C. C. J., a Chinese girl of 8 was admitted on November 11, 1935, for underdevelopment since birth and polyuria, polydipsia and pain in the lower extremities since 1933. The parents were both 40 years of age, living and well. The patient was the eldest

child. Three younger sisters were healthy. The patient was born spontaneously at full term. She was breast fed until the end of the first year when a diet of cereals, vegetables, fish and chicken was substituted. She disliked pork and eggs, and no cod liver oil was received. She began to walk and to talk after her first birthday. Her first tooth erupted in her 13th month. She was considered to be small and inactive.

She had three attacks of "convulsions" together with fever in her 3rd year of life. In the spring of 1933 she was sick with scarlet fever which was complicated by otitis media and later by acute glomerulonephritis. She had general edema for some time. Her urine was always pale. After the attack of scarlet fever, urination became more frequent, 4–5 times in the day time and 1–2 times at night and each voiding was large. She began to drink more than usual. Pain in the legs and feet was frequently complained of. No apparent deformities were observed by the parents. The child failed to gain in height and weight. She was in school for a year in 1934. She had measles in the spring of 1935 and she stopped schooling since.

Physical examination. The patient was markedly underdeveloped and undernourished, 10.9 kg. in weight and 95.8 cm. in height. She was intelligent and suffered from no apparent distress. The skin was dry and scaly. The skull was of normal contour, fontanelles and sutures being closed. The ocular fundi were normal. The right ear canal was filled with mucoid discharge and the drum perforated. The left ear was normal. The tonsils were enlarged. There was no general lymphadenopathy. The neck organs were normal. The chest was slightly prominent on the left side. No rosaries or Harrison's grooves were demonstrated. The lungs were clear. The heart was not enlarged, the left border of dullness being 6 cm. from the midline in the 4th space. There was a systolic thrill at the apex which later disappeared. A loud systolic murmur was heard over the whole precordium. The blood pressure measured 100/60. The abdomen was soft, the tip of the spleen and the edge of the liver being just palpable. The extremities showed knock-knee, enlarged wrists and ankles. The spine was normal. There was no edema of legs. Tendon reflexes were normal.

Roentgenological examination showed normal findings in the skull except for a delay in dental development corresponding to that of a child of about five years. Both the deciduous and permanent teeth were well calcified. The bones of the extremities showed marked general osteoporosis with coarse trabeculation. The spaces between the epiphyses and the diaphyseal ends of the long bones at the wrists, ankles and knees were increased, particularly at the wrists. The diaphyseal ends of the radii and ulnae were flared and irregular, with a zone of marked rarefaction, about 5 mm. in width, adjacent to the margin. The radii and ulnae were so decalcified that no definite cortex could be made out in most portions. The ribs, clavicles and pelvic bones were likewise markedly osteoporotic with coarse and irregular trabeculation. Plain film of the abdomen showed no calcified shadows in the kidney regions.

Laboratory findings. The blood counts gave R.B.C. 4,680,000, Hgb. 13.5 gm., W.B.C. 11,350 and normal differential formula. The urine specific gravity was low. One plus albumin and variable amounts of glucose were constantly present. Acetone bodies were present on two occasions. The average of two Addis' urine sediment counts showed a specific gravity of 1.014 and a protein content of 0.224 gm., granular casts 261,000, hyaline casts 16,000, W.B.C. 417,000 and R.B.C. 65,000 in 12 hours. P.S.P. renal function test showed 23% excretion in 2 hours. Urea clearance was 24.3% of normal. Fasting blood sugar was 83 mg. %, N.P.N. 50 mg. % and CO_2 combining power 30 volumes %. The glucose tolerance curve was essentially normal. The plasma proteins were 3.17% albumin, and 1.92% globulin. The serum calcium was 9.02 and inorganic phosphorus 3.86 mg. %. The plasma chloride was 106 M. eq. per liter. The plasma cholesterol was 139 mg. %. The blood Wassermann test was negative. The stool contained ova of ascaris. The basal metabolic rate was +20.7% (oral temperature 37.7°C.). The electrocardiogram showed normal mechanism except for a P-R interval of 0.20 second. Intelligence test showed an I.Q. of 100.

Course in Hospital Through the courtesy of Dr Charles McKhann and F T Chu, the patient was transferred to the metabolic ward for study on November 20 After a preliminary observation on the low and the high calcium regimes the effect of Vigantol was determined Since the calcium and phosphorus balances failed to improve under the influence of vitamin D, an interfering focus of infection was suspected Tonsillectomy was done on January 3, 1936 Metabolic observation was repeated after the operation Ultraviolet irradiation, in addition to vitamin D, was employed, again without any effect The patient complained of anorexia and vomited several times Finally Vigantol was administered intramuscularly from March 21 to April 10 The results were likewise negative Throughout the patient's stay in the hospital from November 11, 1935 to April 15, 1936, she made only very slight gain in weight and no gain in height Her skeletal condition remained unchanged Her kidney function also remained low on repeated examinations The patient was discharged on April 15 She failed to return to the clinic for follow-up

Case 2 P Y L , a Chinese Mohammedan girl of 17 first came to the orthopedic clinic in August 1934 complaining of pain in the lower extremities and knock-knee for more than 2 years She was born spontaneously but prematurely at 7th month in a poor family She was breast fed during the first year with supplementary feedings with rice porridge and other starchy food She never received cod liver oil Her diet after the first year consisted of cereals and vegetables Animal food was lacking Her general health was delicate, but no important illnesses in her childhood were recalled She had one attack of febrile illness at 11 which was not accompanied by sore throat or skin eruption and which subsided in 10 days Both parents were living and well Her mother had altogether 7 pregnancies The first two pregnancies ended in early abortion The patient was her first child The subsequent 4 children all died in infancy from diarrhea, convulsion or smallpox There was no family history of tuberculosis

For more than two years the patient had noticed slight vague pain in both knees, particularly noticeable on standing or walking She also noticed that her feet failed to meet each other when her knees were in contact She could walk only slowly and her gait became awkward and unsteady Examination in the orthopedic clinic showed that the girl was pale, underdeveloped and poorly nourished Both knees turned inward especially the left The leg and the thigh made an angle of about 150–160° on the lateral aspect of the knees The knee joints were not red, swollen or limited in motion There was no bowing of the legs The hips and spine were normal The serum calcium was 7 3 and inorganic phosphorus 4 0 mg % The patient disappeared from the clinic before examination was complete

On April 1, 1936 the patient returned in much worse condition and was admitted to the orthopedic service During the interval of two years she gradually had more pain in the lower extremities In the last year a heavy sensation and then pain in the lumbar region and pelvis set in In the course of time walking and even standing became difficult She could only sit in bed and kept indoors most of the time In the last six months pain appeared in the shoulder joints and the upper extremities became weak Finally she was completely bed ridden and could not even sit up Frequently she had severe twitchings of the muscles of the lower extremities, particularly at night She had no disturbance of urination, never puffiness of face or edema of legs There was no visual disturbance Her menstruation started at 13 with regular cycles The flow became less in the last two periods

Physical examination showed an underdeveloped and poorly nourished girl weighing 25 0 kg and lying in bed helplessly with her lower extremities flexed at the hips and knees Her skin and mucous membranes were pale There were slightly to moderately enlarged lymph glands in the neck, submaxillary regions and axillae The eye-grounds showed only slight retinal edema and anemia The neck was normal and the thyroid was not enlarged The thorax was asymmetrical, left upper being fuller with slight bulging of

the upper part of the sternum. The breasts were poorly developed. The lungs were clear. The heart was normal in size. There was a loud blowing systolic murmur at the pulmonic area. No thrill or diastolic murmur was made out. The blood pressure was 90/56. The abdomen was soft and no organs were palpable. The external genitalia were normal, but pubic hair was scanty. The upper extremities presented no deformities. The lower extremities showed marked muscular atrophy with coxa vara, knock-knee and inverted feet. Active motion of the hip and knee joints was greatly impaired. Passive motions were possible except abduction and adduction at the hip and extension at the knees, which were accompanied by muscle pain. The pelvis showed marked rostration of the symphysis pubis. The sacrum was bent backward. Pelvic measurement showed a transverse outlet of 6 cm. There was no demonstrable bone tenderness. Tendon reflexes were all active. Chvostek and Trousseau signs were negative.

Radiological examination of the skeletal system showed that the pelvis was markedly deformed, with forward protrusion of the acetabulae, folding of the iliac blades, beaking of the symphysis pubis and forward protrusion of the lumbosacral prominence. Both ilia and ischia showed cystic bone absorption. Same changes were also seen in the heads and the greater trochanters of both femora. The spine showed marked lordosis of the lumbosacral region, slight biconcave deformity of the vertebral bodies with increased intervertebral spaces. The thoracic cage was flattened antero-posteriorly and assumed a slanting fashion. The ribs were osteoporotic. The long bones were moderately osteoporotic throughout. The apiphyses were fused and the joints were normal.

Laboratory examination. The urine had a low and fixed specific gravity and one plus albumin reaction. The sediments were normal. Addis' count showed a urine specific gravity of 1.010, 0.19 gm. of protein, 2,000 hyaline casts, 72,000 R.B.C. and 6,786,000 W.B.C. and epithelial cells in 12 hours. P.S.P. test showed 6% excretion in 2 hours and urea clearance averaged 12.4% of normal. Stool contained cysts of E. coli and ova of trichocephalus. The blood count gave R.B.C. 2,610,000 and Hgb. 8.7 gm. W.B.C. and differential counts were normal. The blood Wassermann test was negative. The serum calcium was 7.3 mg.%, inorganic phosphorus 4.2 mg.%, and phosphatase 29.1 units. The blood N.P.N. was 86 mg. per cent and CO_2 combining power 51.0 volumes %. Plasma proteins consisted of 3.97% albumen and 3.03% globulin. The B.M.R. was −13.4%. Cystoscopy showed normal findings. Retrograde pyelogram revealed that the kidney shadows were very small, approximately 3 x 5 cm., the right slightly larger than the left. The pelves and calices were proportionally small. The ureters were normal. No stone was seen. The urine culture was sterile.

The patient's general condition and the contracture of the lower extremities were considerably improved following admission. She cooperated very well during the period of metabolic observation. She developed symptoms and signs of tetany when she received disodium phosphate solution from May 11 to 18. They disappeared as soon as the phosphate medication was discontinued. Vigantol administration failed to influence her calcium and phosphorus metabolism. The patient was discharged on June 13, 1936, in fairly good condition but with the blood picture and kidney function unimproved.

The patient remained fairly well after discharge for only two weeks and then general aching returned. She had one attack of twitching of the lower extremities in the evening July 14, 1936. She could not walk and was confined indoors throughout the summer. She was readmitted on October 1, 1936 for further study. Physical findings remained essentially the same as during the previous admission. Her eyegrounds remained negative and blood pressure measured 104/70. Skeletal deformities showed no changes. Signs of tetany were absent. Urinalysis and blood counts were the same as before. P.S.P. excretion amounted to 3.5% in 2 hours and urea clearance averaged 9.0% of normal. The blood N.P.N. was 86 mg. per cent, CO_2 combining power 30.0 volumes per cent. Plasma albumin was 2.88% and globulin 3.42%. The serum calcium was 7.24 mg.%. inorganic phosphorus 4.82 mg.% and phosphatase 28.4 units.

During the second admission, the influence of alkali and acid on her calcium and phos-

phorus metabolism and serum electrolyte balance was studied. The patient had tetany and anorexia. Frequent nausea and vomiting made quantitative studies difficult. The administration of sodium bicarbonate, while aggravating the tetany, was followed by a decrease in the acidosis and azotemia with improvement of the appetite. Ammonium chloride in small doses relieved the tetany to some extent, but aggravated the bone ache, acidosis, azotemia and anorexia. Frequent intravenous injections of calcium gluconate resulted in temporary improvement. However, the kidney function became progressively worse, azotemia and acidosis becoming more marked. Convulsions were noted frequently. The patient became drowsy and finally unconscious on February 23, 1937. She was discharged in critical condition at the request of her mother for religious reasons on February 25, 1937. She died at home 5 days later. No autopsy was obtained.

Case 3. M. S. T., a Chinese married woman of 34 was first admitted to the obstetrical service on May 19, 1938, for pregnancy of 5 months, repeated bleeding from the rectum and general bone aching. The patient was apparently healthy in her childhood and before marriage. She was married in 1924 and went through two full term pregnancies in 1925 and 1928 respectively without any complication. Both children were living and well. Her third pregnancy ended in abortion at the third month in 1931. She began to have bleeding after bowel movement shortly afterwards. Bleeding continued at frequent intervals and the amount of blood loss was considerable. Gradually pallor, palpitation of heart and shortness of breath developed. In 1934 she had her fourth pregnancy and in the 6th month of gestation she developed marked anasarca. Her urine was scanty. She had palpitation of heart and shortness of breath on exertion. General bone aching and spasm of hands were also noticed. The pregnancy continued to full term and a living child was delivered spontaneously. The infant died of convulsions two days after birth. Edema, palpitation, dyspnea, bone aching and spasm of hands all subsided gradually after the parturition. The same symptoms recurred in the third month of her fifth pregnancy in 1936 and persisted till one month after a full term spontaneous delivery. The child again died but 6 days after birth. Her sixth pregnancy began in December 1937 and in February 1938 general bone aching returned and gradually became more marked so that she could not walk in the last 4 months prior to admission. Bleeding per rectum persisted and became worse, large blood clots being passed with each movement. Pallor, palpitation of heart and dyspnea on exertion also became worse. Puffiness of face and edema of legs and feet were noticed. Muscular aching and numbness of extremities were also complained of. Her diet consisted of cereals, vegetables, soybean products and occasionally meat. She took one egg every day during the second half of each of her pregnancies. Her husband was healthy and denied venereal disease.

Physical examination on admission showed that the patient was very pale and weak. Her face was puffy with a "butterfly" pigmentation. Slight pitting edema was present over the legs and dorsum of feet. Chvostek and Trousseau signs were both positive. The right cervical, both axillary and left inguinal lymph nodes were palpably enlarged. No lenticular opacities were noticed and ocular fundi showed only anemic changes. The ears, nose and throat were normal. The thyroid was not enlarged. The chest was normal in contour and free from rib tenderness. The lungs were clear. The heart was considerably enlarged with a blowing systolic murmur all over the precordium. No diastolic murmur was heard. The rhythm was regular. The blood pressure was 106/64. The abdomen was distended by the gravid uterus, the fundus of which was 1 finger breadth below the umbilicus. The liver was enlarged, the edge being 2 cm. below costal margin. The spleen was not palpable. Spine and extremities were free from deformities. There was no adductor muscle spasm. The joints were free. All tendon reflexes were present but low. No sensory disturbance was found. The pelvis was symmetrical and not contracted and its measurements were normal.

Laboratory examination. The blood count showed severe anemia, Hgb. 2.7 gm. and R.B.C. 1,420,000. W.B.C. count was 7,800 and differential formula included 84% neutro-

phils. Reticulocyte count was 1.8%. Hematocrit was 11.3%, giving a mean corpuscular volume of 80 cubic micra, mean corpuscular hemoglobin 19 micro-micro-grams and mean corpuscular hemoglobin concentration 24%. The urine contained one plus albumin with a low specific gravity 1.007. Urinary sediments contained variable number of white blood cells, occasionally in small clumps. There were no red blood cells or casts. Renal function test showed 15% excretion of P.S.P. in two hours and the urea clearanec averaged 16.4% of normal. Urinary sediment count by Addis' technique gave granular casts 6,600, R.B.C. 600,000, W.B.C. 50,700,000 in 12 hours. The stool was negative for ova and parasites. The blood Wassermann test was negative. Blood chemical findings were: N.P.N. 50 mg.%, CO_2 combining power 33.8 volumes %, serum calcium 4.2 mg.%, inorganic phosphorus 3.0 mg.%, plasma albumin 3.60% and globulin 3.98%. Gastric analysis showed achlorhydria before and after histamine injection. X-ray of the pelvis and spine showed slight degree of rarefaction of all the bones and slight biconcave deformity of the lumbar vertebrae. Proctoscopic examination revealed external and internal hemorrhoids.

On account of the severe renal insufficiency and marked anemia it was considered undesirable to allow the pregnancy to go on. Termination of pregnancy by vaginal hysterotomy was done on June 2, 1938. The rectal bleeding stopped on bed rest. Calcium lactate, iron, cod liver oil and ultraviolet irradiation improved her anemia and stopped the tetany. When she was discharged on June 17, 1938, her R.B.C. count was 2,040,000, Hgb. 7.0 gm., serum calcium 6.7 mg. and inorganic phosphorus 3.5 mg.%. Her kidney function did not show any improvement.

Second admission. Throughout the summer, 1938, the patient could barely walk with a stick. She preferred to stay in bed most of the time. Pain in the lower extremities and back gradually returned and became worse with the onset of the winter. She was readmitted on January 17, 1939, to the metabolism ward where the first course of study was made. Physical examination on readmission showed that the patient weighed 40.8 kg. She had mild bone tenderness all over, with pain in the lumbar region on moving in bed. No deformities were found in the skeleton but the right shoulder was limited in abduction. Chvostek and Trousseau signs were negative. The eyegrounds showed slight arteriosclerotic changes. The right cervical lymph node was larger. Otherwise, the physical findings were essentially the same as on the first admission.

X-ray of the pelvis, spine and long bones showed only slight osteoporosis. The lungs were clear but the cardiac area measured 57% oversized. The skull was somewhat small and the inner and outer tables were moderately thickened.

The blood picture was that of moderate anemia, Hgb. 7.5 gm. and R.B.C. 2,260,000 and slight leucocytosis, W.B.C. 11,550. The urine findings were the same as before. P.S.P. excretion decreased to 2.5% in 2 hours and urea clearance averaged only 9.8% of normal. The blood N.P.N. increased to 96 mg.% and CO_2 combining power decreased to 22 volumes %. The serum calcium was 5.56 and inorganic phosphorus 3.46 mg.%. Gastric analysis showed persistent achlorhydria. The electrocardiogram showed normal findings. The basal metabolic rate was +12.7%.

Plain x-ray film of the abdomen showed small kidney shadows and retrograde pyelogram revealed normally filled calices and pelvis. They were small but proportional to the size of the kidney.

Ever since readmission in January 1939 the patient ran an irregular fever, sometimes as high as over 40°C. The cause of the fever was never well understood. Repeated blood and urine cultures were negative. Blood smears were free from parasites. Blood agglutination tests were negative. The enlarged gland on the right side of neck became softened and finally ruptured in April. A discharging sinus was formed and was slow in healing. Biopsy of the sinus tissue showed tuberculosis. The patient continued to have bleeding per rectum and hemorrhoidectomy was done on June 8, 1939. With large doses of calcium and iron the patient finally was much improved and the fever subsided in July. She was discharged on July 22, 1939, with a serum calcium of 8.68 and inorganic phosphorus of 1.67 mg.%. Anemia and kidney function remained unchanged.

Third admission The patient remained fairly well throughout the summer and she was admitted for the third time for further metabolic observation on October 2, 1939 Physical findings were the same as on discharge in last July She remained pale and weak The discharging sinus on the right side of neck had healed Few glands remained palpable beneath The heart was still somewhat enlarged The blood pressure measured 92/60 Slight pitting edema of the legs was present Slight tenderness over the lumbar spine, iliac regions and lower ribs was demonstrated Chvostek, Trousseau and Erb's signs were negative X-ray of the long bones, pelvis and spine gave no new findings besides slight general osteoporosis The urine contained traces of albumin, some W B C and occasional granular casts but no R B C Only a trace of P S P was excreted in 2 hours Urea clearance averaged 8 4% of normal The blood count reported R B C 1,670,000 and Hgb 5 4 gm The blood N P N was 68 mg % and CO_2 combining power 23 5 volumes % The serum calcium was 5 22 mg %, inorganic phosphorus 6 56 mg % and phosphatase 6 77 Bodansky units The plasma proteins contained 3 86% albumin and 2 82% globulin Sternal bone marrow smear showed slightly active normocytic erythropoiesis and moderately active granulopoiesis Gastric achlorhydria remained unchanged

The patient remained afebrile throughout her stay in the hospital from October 1939 to May 1940 She cooperated very well in the study and consumed a 1,500 calorie diet quantitatively After two periods of low calcium intake her serum calcium dropped to 4 67 mg % and inorganic phosphorus increased to 7 25 mg % Signs of tetany were elicited but disappeared promptly after the calcium intake was raised The serum calcium was raised by the high calcium intake and further raised by the administration of iron Mild degree of bone pain and backache persisted Slight bleeding from her external hemorrhoid also continued off and on With the administration of A T 10 her serum calcium was raised to 9 18 mg % and she had the best calcium retention Her bone pain and backache also definitely improved and almost entirely disappeared After the effect of A T 10 on the calcium and phosphorus metabolism was over, Vigantol was given another trial but without apparent benefit Lastly she was given large doses of iron again and she was discharged in good condition on May 25, 1940

The patient was regularly followed up since discharge and her general condition remained more or less stationary She has been on large doses of calcium and iron most of the time When she was seen last on June 19, 1941, she complained of more backache X-ray of her skeleton revealed slightly more general rarefaction The serum calcium was 6 46 mg % inorganic phosphorus 5 03 mg % and phosphatase 5 67 Bodansky units Blood N P N remained high, 86 mg per cent, and CO_2 combining power was 31 9 volumes per cent There were no signs of tetany

Case 4 K C C, a Chinese unmarried woman of 20 was admitted on May 14, 1940, with the chief complaint of generalized bone pain of 13 months' duration The patient was born and brought up in Shansi province She had measles in early childhood and otorrhea on the left side for one month at 15 Frequent dull headache was complained of since early childhood There was no history of scarlet fever or tonsillitis She never had urinary disturbance or edema She completed primary school at 16 and then she stayed home learning household duties Her diet was fairly adequate and sun exposure was available In September 1938, on account of war she became a refugee living for several months in crowded quarters and on insufficient food In November 1938 she began to experience a cold sensation in both thighs at night In January, 1939, she developed pain in the ribs, noticeable on deep breathing and on pressure Then pain was noticed in the ankles on walking Tinnitus in both ears was noticed and her vision became impaired particularly at night In March 1939 pain was also present in the popliteal space, trochanteric region and back She had difficulty in walking and in straightening her back, obliging her to stay in bed Her appetite was impaired She became thin and pale In August 1939 she developed moderate pitting edema of feet which subsided in October Occasionally she had evening fever and night sweat She never had muscle spasm, twitching or cramps Her

menstruation which started at the age of 16 with regular cycles became irregular and scanty in the last few months. Her family history was negative.

Physical examination revealed a well developed and fairly well nourished girl. She looked pale, and could hardly sit up in bed. The skin was dry with fairly marked follicular hyperkeratosis over both arms, less marked over both thighs, abdomen and back. There was no general lymphadenopathy. Her head organs were normal except for anemia of optic discs and chronic chorioretinitis. Neck organs were normal but the thyroid appeared full. The chest was symmetrical with well-developed breasts. Some tenderness was present in the lower ribs. The lungs were clear. The heart was normal and the blood pressure measured 106/74. The liver was not palpable and the spleen was barely felt. The spinal column was straight and the pelvis was not deformed. The upper and lower extremities were free from deformities and the joints were normal. Slight to moderate tenderness was present over all the bones. Tendon reflexes were normal. Chvostek and Trousseau signs were absent. There was some tenderness in the calves and thigh muscles.

Roentgenological examination showed that the pelvis was not deformed. The sacrum was slightly anteriorly angulated and the pelvic opening was slightly narrowed anteriorly. The pelvic bones showed osteoporosis with coarse striations. A pseudo fracture was present in the superior ramus of left pubic bone. The spine showed general osteoporosis and coarse striation without any deformity. The long bones of the upper and lower extremities were also osteoporotic with coarse trabeculae. There was no gross deformity. The epiphyseal lines of both radii and ulnae were still visible.

Laboratory examination. Urinalysis showed trace to one plus albumin and a normal sediment. Urine concentration test revealed a maximal specific gravity of 1.015. Phenolsulphonephthalein test showed 7.5% excretion in 2 hours. Blood count reported R.B.C. 2,510,000, Hgb. 6.0 gm. and W.B.C. 5,000 with normal differential formula and smear. Hematocrit studies showed microcytic normochromic anemia. The platelet count was 277,000. The stool was normal. The blood Wassermann test was negative. The blood N.P.N. was 67 mg.%, CO_2 combining power 29 volumes %. The serum calcium was 8.9 mg.%, inorganic phosphorus 4.4 mg.% and phosphatase 11.6 units. The plasma proteins consisted of 3.81% albumin and 2.51% globulin. The serum vitamin A was 12 international units and carotinoid 71 gamma per 100 cc. The basal metabolic rate was −12.4%. Gastric analysis showed no free acid in the fasting specimen and 22 units of free acid after histamine. Cystoscopy showed normal urinary bladder and the catheterized urine was sterile on culture. Retrograde pyelogram showed small kidney shadows and the pelves and calices were small but well outlined. X-ray of the chest showed normal lungs with a dense calcified nodule in the left middle area. After a short period of preliminary metabolic observation on the metabolism ward she was discharged improved on June 16, 1940.

After discharge the patient was seen regularly in the out-patient department throughout the summer 1940. Although backache and pain in the legs were still present she was able to get up and walk around for a few steps. She complained of palpitation and dyspnea. Her hemoglobin decreased to 4.4 gm. and the heart was slightly enlarged. She developed fever again in August, with poor appetite, constipation and amenorrhea. She was readmitted on October 12, 1940.

The patient presented essentially the same physical findings as on discharge except that her heart was slightly enlarged. The eyegrounds showed no new findings apart from the chronic chorioretinitis. The blood pressure remained normal (110/70). No skeletal deformities were demonstrated. Roentgenological survey of the skeleton revealed no new findings. Urine contained one plus albumin and normal sediment. Phenolsulphonephthalein excretion was only 3% in two hours and maximum urea clearance measured 9.6% of normal. The blood contained only 3.5 gm. of hemoglobin and 1,270,000 R.B.C. Blood N.P.N. was 83 mg.% and CO_2 combining power was 28.2 volumes %. The serum calcium was 8.52 mg.%, inorganic phosphorus 4.10 mg.% and phosphatase 10.76 units. Plasma proteins consisted of 4.59% albumin and 2.60% globulin.

On account of the severe anemia and an unexplained fever two blood transfusions of

400 cc. each were given shortly after readmission. Fever subsided within one week and the anemia gradually improved and continued to improve afterwards so that by the end of May 1941 the blood hemoglobin was 9.3 gm. and R.B.C. 3,700,000. However, the kidney function remained poor throughout. Regular metabolic study commenced on October 19. When the phosphorus intake was raised by the administration of trisodium phosphate the patient complained of numbness of face and extremities and her bone pain was aggravated. Chvostek and Erb signs became positive. All symptoms of tetany promptly disappeared on discontinuation of the phosphate. When a high phosphorus diet, which was also high in protein, was given, not only tetany and exacerbation of bone pain, but headache and anorexia were also present. She failed to finish her diet. The blood N.P.N. increased to a maximum of 150 mg.% and the CO_2 decreased to only 13.5 volumes per cent. All the symptoms gradually disappeared after the high phosphorus diet was stopped. Vitamin D in the form of Vigantol did not affect the patient appreciably. Although A.T.10 exerted a favorable influence on the calcium and phosphorus metabolism, there was relatively little subjective improvement. A bone biopsy done on the left tibia on April 8, 1941, showed typical changes of osteomalacia. Her appetite was impaired after the operation. On April 25 the patient was given 1,000,000 i.u. of vitamin D_2 by mouth and very slight, if any, improvement was obtained. Since May 23 iron ammonium citrate was administered with slight subjective improvement. Throughout the entire period of hospitalization the patient continued to put on weight. This increased from 42 to 52 kg. in the course of 8 months. The patient was discharged improved on June 14, 1941. She remained in fairly good condition when seen in the outpatient department in September.

Case 5. C. C. C., a Chinese Mohammedan boy of 20 was admitted to the orthopedic service on October 29, 1940, with the chief complaint of pain and swelling of the knees, progressive deformity of the lower extremities and backache of 8 years' standing. The patient was born into a poor family. His diet was poor, lacking in fresh vegetables and animal food. He was always thin in early childhood but his development was normal up to 1932. In the spring of 1932 his right knee was hurt during a fall. Apart from pain, the knee showed no external wound or impaired motion. Two weeks later it became swollen and painful on walking. The patient continued to have a limping gait for one year. Gradually pain in the lumbar spine was noticed, particularly on walking. This persisted till 1935 when the patient began to experience weakness in his lower extremities and back. Walking became difficult and he tumbled easily. In 1936 weakness became more marked and he could walk only with the help of a stick. Gradually he had difficulty in straightening his back and his lower extremities. Pitting edema was noticed in both feet and ankles. This would disappear on rest. He never noticed puffiness of face. There was frequent dizziness. He had no urinary disturbance. In July, 1940, the patient fell down again, injuring his left knee. Immediately he could not move his left lower limb and the left knee was swollen and painful. A dislocation was suspected and after manipulation by a native bone-setter the whole left thigh became swollen. Walking was impossible. Ever since his first accident in 1932 the patient's appetite had always been poor and he failed to grow any more. On the contrary, he became emaciated. He had diarrhea for one month in 1937. In 1938 he had night blindness which was cured by taking sheep liver. He had measles in early childhood. There was no history of scarlet fever or tonsillitis.

Physical examination revealed that the patient was much underdeveloped and markedly emaciated, weighing only 26 kg., and having slight fever. He was completely bedridden, unable even to sit up on account of pain in the back, hips and knees together with contractures of the lower limbs. The skin was dry and rough. The submaxillary lymph-glands were enlarged. The skull was of normal contour. The ocular fundi were normal. The conjunctivae were free from xerosis and the cornea was clear. The chest showed prominent costochondral junctions of the lower ribs on both sides. There was no deformity of the sternum or Harrison's groove. The lungs were clear. The cardiac findings were normal. The blood pressure measured 84/64. The abdomen was normal. The external

genitalia were underdeveloped. The spine showed left-sided scoliosis of the lower dorsal and lumbar segments. The lumbar spine was fixed, no lateral or antero-posterior motion being possible. Paravertebral muscles were spastic. The upper extremities showed marked muscular atrophy and the bones were slender with enlargement at the wrists. The fingers were slender and could be hyperextended to an unusual degree at the metacarpo-phalangeal and interphalangeal joints. The lower extremities were held in flexion deformity at the hip and knees. There was genu valgum. Both knees were moderately swollen with floating patella. A fracture was present at the lower third of the left femur, the upper segment overriding the lower giving rise to a prominence in the suprapatellar region. Tenderness and grating sensation were present. The ankles were enlarged but there was no limitation of motion. The pelvis was tilted, the right side being higher than the left. Tendon reflexes were normal. Chvostek sign was not obtained. Trousseau and Erb signs were positive.

Roentgenological examination of the skeleton showed very extensive osteoporosis in all the bones of the spine and pelvis. The trabeculae were coarse and irregular. The secondary trabeculae disappeared, resulting in a "washed out" appearance of the bones. The articulating facets were cloudy. The heads of both femora were flattened. Similar osteoporosis was present in the bones of both knees. The joint surface was intact. There was an old fracture of the lower end of the left femur. Marked patchy osteoporosis was present in the humeri, radii and ulnae. Irregularity and cupping were seen in the diaphyseal ends of the radii at the wrists. The skull showed mottling in the parietal region and thinning of the vault. X-ray of the chest showed no important changes in the lungs but a substernal shadow was present. Plain film of the abdomen showed no radiopaque stones in the kidney regions.

Laboratory examination showed that the urine contained one plus albumin but no abnormal sediment. The maximal specific gravity during a concentration test was only 1.013. Phenolsulphonephthalein excretion was 7% in two hours and maximum urea clearance averaged 13.2% of normal. The blood contained 9.2 gm. of hemoglobin, 3,410,000 R.B.C., 7,200 W.B.C. and 393,000 platelets. Hematocrit studies showed normocytic anemia. Sternal marrow smear showed normoblastic erythropoiesis. The blood Wassermann test was negative. The blood sedimentation rate was very rapid. The serum N.P.N. was 50 mg.%, CO_2 combining power, 28.2 volumes %, calcium 8.1 mg.%, inorganic phosphorus 3.6 mg.%, phosphatase 16.4 units, plasma albumin 3.63% and globulin 3.76%. The stool contained ova of ascaris. The basal metabolic rate was +1.2%. The liver function by the bromsulphthalein test and galactose tolerance test was normal. Gastric analysis showed achlorhydria. The joint fluid aspirated from the right knee was serofibrinous, containing 91,440 W.B.C. with 98.5% P.M.N. Smear and culture showed no organism. Cystoscopy showed normal urinary bladder. The catheterized urine was sterile on culture. Retrograde pyelogram revealed small and contracted kidneys. The kidney pelves and calices were well filled and showed no dilatation.

Shortly after admission the patient developed a relapse of his dysentery which subsided promptly. Stool examination revealed no amoeba or B. dysenteriae on culture. The patient was, through the courtesy of Drs. C. M. Meng and H. C. Fang, transferred to the metabolism ward for study. Except for frequent dizziness and irregular appetite, the first part of the metabolic observation was satisfactorily carried out. After a high calcium regime he was given Vigantol which, however, failed to bring about any change in his calcium and phosphorus metabolism. When a high phosphorus diet was next given the patient was immediately made very sick complaining of dizziness, headache, marked anorexia, nausea and vomiting. The blood N.P.N. was raised to 86 mg.% and CO_2 decreased to 30.2 volumes %. Chvostek and Trousseau signs remained negative. All symptoms promptly improved when the diet was discontinued. Vitamin D_2, 1,000,000 i.u. was given by mouth on February 4, 1941, and the improvement of the calcium and phosphorus metabolism was only slight. The joint effusion of the knees promptly subsided in November. He had a spontaneous refracture of the lower third of the left femur in February which

was immobilized by a long leg splint He had but little pain and a transient fever Following the fracture the left knee became swollen again and aspiration was repeated On April 5 the patient was given A T 10, but unfortunately before the effect was manifest the medication had to be discontinued on account of fever, headache and poor appetite Irregular fever continued without any adequate explanation Extreme anorexia, nausea and frequent vomiting made quantitative study impossible Fever became higher and some chilliness was also noticed Anemia became more marked and a leucocytosis was present Finally on May 9, the patient presented symptoms and findings of a rectal abscess The latter subsided before surgical intervention was attempted Fever and leucocytosis persisted Both knees and ankles became swollen and painful again Headache and anorexia continued The blood N P N went up to 120 mg % and CO_2 dropped to 15 1 volumes % Blood transfusion, glucose infusion and calcium gluconate injections failed to improve the condition He was discharged in very poor condition at the request of his family on May 29, 1941 He died at home few days after discharge

MASSED METABOLIC DATA

TABLE 1

Partition of phosphorus excretion in relation to the ratio of calcium to phosphorus intake and renal function

CASE	LOW Ca/P INTAKE			HIGH Ca/P INTAKE			SERUM		RENAL FUNCTION	
	Period no.	Diet Ca/P	P output in urine	Period no.	Diet Ca/P	P output in urine	Inorg. P	N.P.N.	P.S.P.	Urea clearance
			% of total output			*% of total output*	*mg.%*	*mg.%*	*% in 2 hours*	*% of normal*
1	1– 2	0.27	76	3	2.23	68	3.97	41	23.0	24.3
2	1– 2	0.29	54	6–7	1.80	43	5.90	85	6.0	12.4
3	47–48	0.43	47	49–52	2.00	34	6.89	90	2.5	8.4
4	1– 4	0.44	40	20–21	2.14	34	4.76	86	3.0	9.6
5	1– 3	0.39	45	4– 6	2.63	32	4.09	64	5.0	13.2
Normal*		0.38	63		1.60	57	3.99	19		

* Data from 12 normal Chinese (38) in whom the urinary phosphorus varied from 57 to 74% of the total output on low Ca/P intake and from 46 to 63% of the total output on high Ca/P intake.

TABLE 2

Effect of increasing phosphorus intake on calcium and phosphorus metabolism and serum calcium and inorganic phosphorus

	REGIMEN*	SOLUBLE PHOSPHATE ADDED			HIGH PHOSPHORUS FOOD ADDED	
		Case 2	Case 3	Case 4	Case 4	Case 5
Period no	Control	6–7	16–17	1–4	8–11	11–13
	High P	8–9	18–19	5–7	12–14	14–15
	Post-control	10–11	20	8–11	15–17	16
P intake, *mg daily*	Control	675	612	618	680	488
	High P	1075	1080	1059	1073	863
	Post-control	675	585	680	569	191
Ca intake, *mg daily*	Control	1207	989	271	336	1356
	High P	1207	980	271	329	1438
	Post-control	1207	979	336	237	1270
Ca/P intake	Control	1 89	1 62	0 45	0 49	2 98
	High P	1 12	0 91	0 26	0 31	1 67
	Post-control	1 89	1 67	0 49	0 42	6 65
P in stool, *mg daily*	Control	412	435	370	436	221
	High P	540	582	338	610	448
	Post-control	407	684	436	350	286
P in urine, *mg daily*	Control	314	218	251	365	175
	High P	418	323	465	339	194
	Post-control	338	257	365	334	170
P in urine, *% total output*	Control	43	33	40	46	44
	High P	44	36	58	37	30
	Post-control	45	27	46	49	37
P balance, *mg daily*	Control	−56	−40	11	−122	92
	High P	117	176	256	124	221
	Post-control	−70	−356	−122	−115	−265
Ca balance, *mg daily*	Control	63	31	−52	−60	149
	High P	114	−12	−9	−62	160
	Post-control	114	−295	−60	−117	82
Serum inorg P, *mg %*	Control	5 96	5 89	1 95	5 00	3 70
	High P	7 25	9 26	8 33	7 11	5 36
	Post-control	6 02	5 24	5 00	6 14	3 86
Serum Ca, *mg %*	Control	5 54	5 46	8 04	7 73	8 31
	High P	4 86	4 14	5 38	6 80	8 40
	Post-control	6 31	4 70	7 73	7 54	8 56
Serum N P N, *mg %*	Control	76	104	97	86	43
	High P	80	84	100	150	86
	Post-control	86		86	100	55

* Figures for intake, stool and urine are averages for the whole of each regimen, and those for serum are for the end of each regimen

TABLE 3

Influence of calcium intake on calcium and phosphorus balances and serum calcium and inorganic phosphorus

CASE	PERIOD NO.	BODY WEIGHT	CALCIUM			PHOSPHORUS		SERUM	
			Intake	Intake	Balance	Intake	Balance	Ca	P
Low calcium intake									
		kg.	*mg.*	*mg./kg.*	*mg.*	*mg.*	*mg.*	*mg.%*	*mg.%*
1	1– 2	10	96	9.6	−18	358	−5	8.45	4.06
2	1– 2	25	161	6.4	−87	554	−26	6.06	6.25
3	47–48	38	415	10.9	−25	962	242	4.74	7.60
4	1– 4	40	271	6.8	−52	618	11	8.04	4.95
5	1– 3	25	186	7.4	−72	479	49	7.84	4.07
Normal*		56	471	8.4	63	1258	96		
High calcium intake									
1	1– 3	10	796	79.6	321	358	13	8.03	3.26
2	6– 7	25	1207	48.3	63	675	−56	5.54	5.96
3	49–52	38	1915	50.4	346	962	146	6.14	4.90
4	20–21	40	1451	36.3	136	678	113	8.14	4.02
5	4– 6	25	1353	54.1	96	515	53	9.11	3.26
Normal*		56	1205	21.5	211	760	3		

* Data from 12 normal Chinese (38) in whom the average serum calcium was 10.07 ± 0.037 mg. %, and inorganic phosphorus 3.99 ± 0.033 mg. % without significant variations in relation to the different levels of calcium and phosphorus intake.

TABLE 4

Serum electrolytes unassociated with acid or alkali administration

CASE		pH	HCO_3^-	Cl	HPO_4^{--}	ALB.	GLOB.	SUM OF ANIONS	UNDET. ANIONS	TOTAL BASE	Na^+	K^+	Ca^{++}	Mg^{++}	N.P.N.
1	Nov. 14, '35		13.4	106	2.3	7.3	5.7						4.5		43
2	Apr. 9, '36	7.33	22.8	103.9	4.1	9.7	5.6	146.1	4.9	151.0	141.2	3.6	2.8	2.9	85
	June 12, '36	7.35	18.6	112.0	3.0	9.7	4.9	148.2	1.7	149.9	138.3	3.6	3.1	2.6	77
	Oct. 7, '36	7.28	16.0	106.0	3.8	10.0	5.4	141.2	2.9	144.1	137.2	3.4	2.7	2.7	
3	Jan. 23, '39	7.25	19.0	104.0	2.8	7.5	7.0	141.3	13.2	154.5			2.5		82
4	Nov. 1, '40	7.24	14.1	110.4	2.9	10.3	5.1	142.8	8.6	151.4	134.9	5.6	4.0	4.0	97
	May 23, '41	7.32	19.8	112.4	2.7	9.9	4.4	150.2	6.5	156.7	135.5	4.5	4.1	3.6	59
5	Nov. 18, '40	7.25	16.5	103.7	2.7	8.7	6.8	138.2	8.9	147.1	138.0	2.9	3.7	2.8	64
	Apr. 17, '41	7.25	12.9	105.3	1.9	8.0	6.7	134.6	12.8	147.4	131.5	4.0	4.7	2.7	68
	May 8, '41	7.10	15.6	101.4	2.2	7.7	4.8	134.2	10.9	145.1					72
	May 22, '41	7.18	12.7	97.2	2.9	5.1	7.0	124.9	13.1	138.0	118.0	3.9	4.2	2.6	131
N*	Average	7.38	28.2	100.4	2.6	10.8	4.8	148.6	3.2	150.0	138.0	3.9	4.9	2.3	27
	Lowest	7.35	25.8	95.5	2.2	9.4	3.8	142.6	0	146.1	135.5	3.3	4.4	1.9	22
	Highest	7.40	31.0	104.6	3.4	12.0	5.9	151.1	7.7	154.7	140.0	4.2	5.5	2.6	34

* 12 normal controls. Figures are expressed in milli-equivalents per liter of serum except pH and N.P.N. which are in mg. per 100 cc. of serum.

TABLE 5

Serum electrolytes after alkali and acid administration

Case 2

	pH	HCO_3^-	Cl	HPO_4^{--}	ALB.	GLOB.	SUM OF ANIONS	UNDET. ANIONS	TOTAL BASE	Na^+	K^+	Ca^{++}	Mg^{++}	N.P.N.	REMARKS
Nov. 9, '36	7.22	11.6	107.0	4.2	8.8	4.4	136.0	9.3	145.3	135.5	3.6	2.3	2.5	168	Control
Nov. 17, '36	7.35	22.0	96.1	4.3	9.7	5.3	137.4	5.8	143.2	137.0	2.8	2.0	1.7	131	80 cc. M $NaHCO_3$ daily Nov. 13–16
Nov. 29, '36	7.32	16.1	101.2	3.5	9.4	5.6	135.8	7.8	143.6	135.4	3.4	2.3	2.1	110	
Dec. 7, '36	7.40	24.8	96.6	3.5	9.9	5.2	140.0	5.4	145.4	139.4	4.0	2.3	2.4	97	40 cc. M $NaHCO_3$ daily Nov. 29–Dec. 10
Dec. 23, '36	7.30	16.8	103.6	4.0	11.6	4.1	140.1	6.7	146.8	137.4	4.2	2.6	2.6	124	
Dec. 31, '36	7.20	8.3	106.7	3.8	9.5	4.9	133.2	6.9	140.1	132.0	3.5	2.4	2.5	142	1 gm. NH_4Cl daily Dec. 23–30
Feb. 22, '37	7.28	14.9	99.9	4.8	6.8	6.1	132.5	12.0	144.5	135.8	2.9	1.8	2.0	148	

Figures are expressed in milli-equivalents per liter of serum except pH and N.P.N. which are in mg. per 100 cc. of serum.

TABLE 6

Summary of results of iron therapy

	CASE 3			CASE 4
	Trial 1	Trial 2	Trial 3	Trial 1
Ferric amm. citrate, *gm. daily*....	6	6	6–12	6
Duration of iron therapy, *days*...	52	56	24	20
Serum inorganic phosphorus, *mg. %*.....	3.70 to 1.00	4.9 to 2.77	3.70 to 2.96	4.60 to 3.94
Serum calcium, *mg. %*.....	5.82 to 9.00	6.14 to 7.87	7.50 to 7.49	8.15 to 8.43
Phosphorus balance, *mg. daily*....	22 to 32	146 to 5	76 to 16	149 to 74
Calcium balance, *mg. daily*.....	67 to 410	346 to 157	216 to 564	238 to 604
Phosphorus intake, *mg. daily*.....	707	929–640	626	602
Calcium intake, *mg. daily*.....	1978	1919	2009	1671
Nitrogen intake, *gm. daily*.....	7.11	8.90–7.97	7.62–7.90	6.61
Nitrogen in stool, *gm. daily*.....	1.70	2.10	1.67	1.36

TABLE 7

Composition of diets in gram per day

FOOD ARTICLES	CASE 1. C. C. J.			CASE 2. P. Y. L.			CASE 3. M. S. T.									CASE 4. K. C. C.										CASE 5. C. C. C.						
	Diet																																
	1	2	3	1	2	2a	1	2	3	4	5	A	B	C	D	1a	1b	1c	2	3a	3b	3c	4	5	6	1	2	3a	3b	4a	4b	5
Wheat flour	50			150	160	150	150	150	100	100	100	100	100	100	100	150	150	150	250	250	75	150	200	150	150	150	200	150		150	200	150
Rice	80	60	50	40	48	50	100	100	60	60	60	100	100	100	100	100	100	100		75	75	75	75	75	50	60	60			60	60	60
Millet				30	21														120									100	100			
Kau mien			20													60	60	60	60	60	60	60	60	60	60							
Kan fen														10	10	10	10	10	10	20	20	20	20									
Ou fen														10	10																	
Graham bread		50																														
Pork		50					30									10	10	10	20	30	30	30	30									
Beef	50		40	40	32	50																				40						
Chicken	40	50	50																					30	30							
Liver, pork												30	30	30	30	20	20	20	40													
Liver, beef					40	50																						30	30			
Liver, sheep																													65	30	30	30
Egg				60	48	30	30	30	30	30	30	60	60																			
Egg white														50	50					50						100						
Milk							200	200	200	200	200	200	200			100	100	100	100		100	100	100	200	200		100	200	200	100	100	100
Klim			30																													
Bean curd										50		25	25	50	50																	
Cabbage, large	50	30	50	40	32	30	50	50				60	110	150	100	200	200	200	200	200	200	200	200			200						
Cabbage, small											50													150	150							
Mung bean sprout	50							50		50						100	100	100	100	100	100	100										
Spinach				50	64		50																100									
Raddish											100				50									100	100							
Turnip						30	50																			100						
Wosun	40																															
Cucumber												50																				
White potato		40	20						100	100	100	100	100	100	150		200	200		200	100	100	100	150		200	200					
Sweet potato				10	8	10						10	20	20	20												50	50	50	50	50	30
Salted turnip		30						25	25	25	25	30	30		10				40									50	50			30
Peanut														50																		
Chestnut	50		50												200	50	50	50	50	50	50	50	50	50	50		200					
Apple	50			100	80	100						100	200	200		150	150	150	150	150	150	150	150	150	150	200	200	200	200	200	200	200
Pear												100	100	100																		
Persimmon																																

Orange							100	200	200	200	200																				100	100	100
Banana		100																															
Butter		10												10	10																		
Peanut butter									30															10	5								
Sesame oil	30	20	20	20	24	30	30	15		5	5	6	8	10	10	30	30	30	30	30	30	30	30	30	30	30		5	5				
Soy bean sauce										10	5	10	15	20	20									10	10			10	10				
Vinegar											20		5			30	30	30	30	30	30	30	30	30	30								
Sugar			20			40	20	20	20	20	20	20	20	20	20	10	10	40	10	20	20	20	20	20	20		10	10	10	10	20	20	
NaCl	2	2	2	2	2	2	4	3			4	2	2	3	4	3	3	3	3	3	3	3	3	1	1	5							
Calories*	901	1022	819	1157	1221	1354	1564	1529	1349	1271	1274	1527	1590	1555	1546	1413	1649	1769	2394	2259	1567	1832	1996	1847	1512	1489	1568	1504	1071	1027	1273	1220	
Calcium† mg	99	92	290	161	207	119	376	333	367	359	458	415	419	252	309	271	330	336	329	210	220	218	294	548	527	185	199	302	287	165	175	228	
Phosphorus† mg	338	440	454	554	675	604	659	602	629	612	653	962	929	642	620	618	690	680	1073	578	482	588	678	691	624	479	488	907	874	418	451	515	
Nitrogen†	5 06	5 54	5 42	0 57	7 62	7 57	7 00	6 70	7 29	6 27	6 10	9 79	8 62	7 69	7 34	6 84	7 14	7 14	11 89	8 94	5 46	7 29	7 80	7 42	0 75	7 63	5 74	8 58	7 38	4 73	5 49	6 01	

* *Calculated from Outlines of Diets of the Peiping Union Medical College Hospital,"* 3rd ed , 1937

† Analyzed values

TABLE 8

Case 1. C. C. J. Effect of vitamin D and ultraviolet irradiation on Ca, P and N_2 metabolism

DATE 1935-36		PERIODS 4 DAY	CALCIUM, MG., DAILY				PHOSPHORUS, MG. DAILY				NITROGEN, GM. DAILY				SERUM MG. %		REMARKS
			Intake	Urine	Stool	Balance	Intake	Urine	Stool	Balance	Intake	Urine	Stool	Balance	Ca	P	
Nov.	21-24	1	96	12	112	− 28	358	286	109	− 37	5.06	4.19	0.54	+0.33	9.02	3.86	diet 1
	25-28	2	96	18	86	− 8	358	265	66	+ 27	5.06	4.04	0.36	+0.66	9.27	4.00	diet 1
	29-2	3	796	18	466	+312	358	236	109	+ 13	5.06	4.17	0.44	+0.45	8.45	4.06	diet 1
Dec.	3-6	4	796	30	922	−156	358	227	184	− 53	5.06	4.23	0.65	+0.18	8.03	3.26	diet 1 Vigantol 30 drops daily
	7-10	5	796	20	586	+190	358	229	119	+ 10	5.06	4.36	0.37	+0.33	8.85	3.40	diet 1 Vigantol 30 drops daily
	11-14	6	796	34	462	+300	358	204	101	+ 53	5.06	4.23	0.34	+0.49	8.45		diet 1 Vigantol 30 drops daily
	15-18	7	796	30	677	+ 89	358	211	145	+ 2	5.06	4.18	0.53	+0.25	8.48	3.01	diet 1 Vigantol 30 drops daily
	19-22	8	796	17	549	+230	358	200	120	+ 38	5.06	4.15	0.45	+0.46	8.26	3.02	diet 1 Vigantol 30 drops daily
Jan.	12-15	9	796	29	532	+235	358	184	142	+ 32	5.06	3.92	0.47	+0.67			diet 1
	16-19	10	796	19	298	+479	358	190	76	+ 92	5.06	4.02	0.28	+0.76	8.88	3.04	diet 1
	20-23	11	796	10	831	− 45	358	204	206	− 52	5.06	4.07	0.59	+0.40	8.90	3.04	diet 1
	24-27	12	796	19	469	+308	358	192	116	+ 50	5.06	3.92	0.37	+0.77			diet 1 Vigantol 60 drops daily
	28-31	13	796	12	715	+ 69	358	183	194	− 19	5.06	4.28	0.57	+0.21			diet 1 Vigantol 60 drops daily
Feb.	1-4	14	796	9	685	+102	358	204	183	− 29	5.06	3.61	0.56	+0.89	9.12	3.02	diet 1 Vigantol 60 drops daily
	5-8	15	796	5	511	+280	358	180	207	− 29	5.06	4.06	0.53	+0.47			diet 1 Vigantol 60 drops daily
	9	16													8.84	3.10	diet 1 Vigantol 60 drops daily
	13-16	17	792	27	534	+231	440	196	198	+ 46	5.54	4.62	0.54	+0.38	9.10	3.02	diet 2 Vigantol 60 drops daily
	17-20	18	792	40	318	+434	440	186	158	+ 96	5.54	4.74	0.39	+0.41	8.90	3.32	diet 2 Vigantol 60 drops daily
	21-24	19	792	33	588	+171	440	208	201	+ 31	5.54	4.97	0.48	+0.09			diet 2 Vigantol 60 drops daily
	25-28	20													8.82	3.42	diet 2 Ultraviolet irradiation
	29-3	21	792	42	963	−213	404	178	382	−120	5.54	4.16	0.98	+0.40			diet 2 Ultraviolet irradiation
March	4-7	22	784	23	416	+345	402	201	158	+ 43	5.13	4.52	0.42	+0.19			diet 2
	8-11	23	690	16	498	+176	454	282	290	+118	5.42	4.72	0.60	+0.10			diet 3
	12-15	24	690	21	418	+251	454	304	154	− 4	5.42	4.46	0.35	+0.61	8.52	3.76	diet 3
	16-19	25	690	0	399	+291	454	297	148	+ 9	5.42	4.82	0.39	+0.21			diet 3
	20-23	26	690	24	550	+116	454	309	198	− 53	5.42	4.92	0.47	+0.03			diet 3 Vigantol 1.2 cc. i.m. daily
	24-27	27	690	21	487	+182	454	324	169	− 39	5.42	4.93	0.49	0	8.64	4.25	diet 3 Vigantol 1.2 cc. i.m. daily
	28-31	28	690	38	519	+133	454	314	203	− 63	5.42	4.42	0.49	+0.51			diet 3 Vigantol 1.2 cc. i.m. daily
April	1-4	29	690	0	490	+200	454	334	161	− 41	5.42	5.05	0.51	−0.14			diet 3 Vigantol 1.2 cc. i.m. daily
	5	30													9.19	4.20	diet 3 Vigantol 1.2 cc. i.m. daily

TABLE 9

Case 2. P. Y. L. Effect of vitamin D, phosphate, acid and alkali on Ca, P and N_2 metabolism

DATE 1936	PERIOD 4-DAY	CALCIUM, MG. DAILY				PHOSPHOROS, MG. DAILY				NITROGEN, GM. DAILY				SERUM, MG. %			REMARKS
		Intake	Urine	Stool	Balance	Intake	Urine	Stool	Balance	Intake	Urine	Stool	Balance	Ca	P	N.P.N.	
April 13-16	1	161	0	184	− 23	554	310	212	32	6.57	4.58	0.88	1.11	6.68	5.78	85	diet 1
17-20	2	161	0	312	−151	554	316	321	−83	6.57	4.78	1.09	0.70	5.94	5.68		diet 1
21-24	3	661	0	602	−41	554	323	333	−102	6.57	5.18	0.96	0.43	6.06	6.25		diet 1
25-28	4	707	0	686	21	675	368	351	−44	7.62	5.70	0.84	1.08	5.56	5.99		diet 2
29-2 (May)	5	707	0	560	147	675	361	263	51	7.62	5.74	0.72	1.16				diet 2
May 3-6	6	1207	0	1212	−5	675	326	486	−137	7.62	5.33	1.00	1.29	5.63	6.85		diet 2
7-10	7	1207	0	1076	131	675	301	348	26	7.62	5.28	0.95	1.39	5.91	6.29	76	diet 2
11-14	8	1207	0	1055	152	1075	402	520	153	7.62	5.65	1.05	0.92	5.54	5.96		diet 2 Na_2HPO_4
15-18	9	1207	0	1130	77	1075	434	560	81	7.62	5.55	1.08	0.99	5.02	6.87	80	diet 2 Na_2HPO_4
19-22	10	1207	0	1015	192	675	369	356	−50	7.62	5.62	0.82	1.18	4.86	7.25		diet 2 Vigantol
23-26	11	1207	0	1170	37	675	307	458	−90	7.62	5.19	1.06	1.37	6.65	6.33		diet 2 Vigantol
27-30	12	1207	0	976	231	675	309	316	50	7.62	5.16	0.92	1 54	6.31	6.02	86	diet 2 Vigantol
31-3 (June)	13	1207	0	1044	163	675	296	321	58	7.62	5.16	1.15	1.31	6.60	5.62		diet 2 Vigantol
June 4-7	14	1207	0	967	240	675	307	294	74	7.62	4.95	1.00	1.67	6.20	6.54		diet 2 Vigantol
8	15													6.54	5.96	110	
Oct. 2																86	
28-31	21	919	0	575	344	604	73	267	264	7.57	3.57	0.92	3.08	5.11	6.25	127	diet 2a
Nov. 1-4	22	919	1	736	182	604	122	276	206	7.57	4.20	0.76	2.61	5.21	5.86		diet 2a
5-8	23	919	1	636	282	604	150	264	190	7.57	5.11	0.52	1.94	5.36	6.61	168	diet 2a
9-12	24	874	4	849	21	552	167	178	207	7.09	5.36	0.96	0.77	4.60	7.25		diet 2a
13-16	25	670	20	340	310	404	179	152	73	4.78	5.47	0.38	−1.07	4.57	7.54		diet 2a $NaHCO_3$
17-20	26	690	8	616	66	453	219	271	37	5.68	5.19	0.48	0.01	4.07	7.45	131	diet 2a
21-24	27	919	1	864	54	604	165	424	15	7.57	4.75	0.82	2.00	4.65	6.27		diet 2a
25-28	28	919	6	543	370	604	149	288	167	7.57	4.50	0.70	2.37	4.75	5.91		diet 2a
29-2 (Dec.)	29	919	0	816	103	604	148	439	17	7.57	4.62	0.92	2.03	4.57	5.97	110	diet 2a $NaHCO_3$
Dec. 3-6	30	919	5	766	148	604	158	392	54	7.57	5.06	0.93	1.58	4.75	5.55		diet 2a $NaHCO_3$
7-10	31	919	4	643	272	604	178	348	78	7.57	4.50	0.88	2.19	4.65	6.02	97	diet 2a $NaHCO_3$
11-14	32	919	7	737	175	604	193	354	57	7.57	4.60	0.75	2.22	5.06	5.74	91	diet 2a
15-18	33	919	12	594	313	604	217	256	131	7.57	5.17	0.52	1.88	6.09	6.26		diet 2a
19-22	34	919	25	700	194	604	235	348	21	7.57	4.94	0.81	1.82	5.99	6.45	114	diet 2a
23-26	35	919	7	620	292	604	204	302	98	7.83	5.44	0.69	1.70	5.14	6.81	124	diet 2a NH_4Cl
27-30	36	904	18	676	210	528	164	314	50	6.88	4.88	0.75	1.25				diet 2a NH_4Cl
31														4.81	6.63	142	

TABLE 10

Case 3. M. S. T. Effect of calcium, phosphate, iron, vitamin D and parathormone on Ca, P and N_2 metabolism

DATE 1939–40		PERIODS 4 DAY	CALCIUM, MG. DAILY				PHOSPHORUS, MG. DAILY				NITROGEN, GM. DAILY				SERUM, MG. %				REMARKS
			Intake	Urine	Stool	Balance	Intake	Urine	Stool	Balance	Intake	Urine	Stool	Balance	Ca	P	Phosphatase*	N.P.N.	
Jan.	23–26	1	620	2	507	+111	613	188	305	+120	6.40	4.31	0.94	+1.15	5.00	4.86		82	diet 1
	27–30	2	629	0	628	+1	611	190	294	+127	6.25	4.16	1.07	+1.02	5.82	4.39	2.05		diet 1
	31–3 (Feb.)	3	640	7	462	+171	579	222	280	+71	5.81	4.28	0.92	+0.61	6.02	5.75			diet 1
Feb.	4–7	4	624	19	392	+213	544	194	304	+46	5.40	4.64	1.08	−0.32	6.60	4.56			diet 1
	8–11	5	856	0	828	+28	486	87	398	+1	5.53	4.14	1.08	+0.31	6.75	4.90			diet 2
	12–15	6													5.84	4.94		86	
	16–27	7–9													5.28	5.82		75	Vigantol 1 cc. daily
	28–3 (Mar.)	10	967	2	753	+212	629	136	405	+88	7.29	4.35	0.94	+2.00	5.47	4.36			diet 3 Vigantol 1 cc. daily
March	4–7	11	967	0	1069	−102	629	152	532	−55	7.29	4.99	1.16	+1.14	5.26	4.96	2.80		diet 3 Vigantol 1 cc. daily
	8–11	12	967	0	881	+86	629	172	471	−14	7.29	4.59	1.24	+1.46	5.38	5.19		105	diet 3 Vigantol 5 cc. daily
	12–15	13	964	0	908	+56	619	177	496	−54	6.29	4.85	1.08	+0.36	5.94	5.91			diet 3 Vigantol 5 cc. daily
	16–19	14	967	0	944	+23	629	193	538	−102	7.29	4.95	1.45	+0.89	4.80	6.28			diet 3
	20–23	15	967	0	932	+35	629	196	448	−15	7.29	4.97	1.50	+0.82	5.06	5.92		104	diet 3
	24–27	16	989	0	866	+123	612	215	396	+1	6.27	4.66	1.15	+0.46	5.55	5.89			diet 4
	28–31	17	989	0	1050	−61	612	220	474	−82	6.27	4.61	1.16	+0.50					diet 4
April	1–4	18	989	1	1037	−49	1105	252	600	+253	6.27	4.25	1.33	+0.69	5.46	5.58			diet 4 phosphate sol.
	5–8	19	970	1	944	+25	1055	394	563	+98	5.34	2.74	1.35	+1.25	4.76	7.77	3.91	84	diet 4
	9–12	20	979	0	1274	−295	585	257	684	−356	5.73	3.82	1.74	+0.17	4.14	9.26			diet 4
	13–16	21	989	1	929	+59	612	158	411	+43	6.27	4.36	1.14	+0.77	4.70	5.24			diet 4
	17–20	22	989	0	986	+3	612	135	488	−11	6.27	4.11	1.25	+0.91	5.23	4.35			diet 4
	21–24	23	1058	1	1108	−51	653	160	503	−10	6.19	4.28	1.48	+0.43	5.31	4.49			diet 5
	25–28	24	1058	3	1014	+41	653	193	440	+20	6.19	4.09	1.25	+0.85	5.09	5.08			diet 5
	29–2 (May)	25	1058	0	892	+166	653	235	358	+60	6.19	3.80	1.16	+1.23	5.15	4.89			diet 5 parathormone 40 units daily
May	3–6	26	1058	0	968	+90	653	250	382	+21	6.19	3.87	1.22	+1.10	5.50	5.39			diet 5 parathormone 40 units daily
	7–10	27	1058	0	995	+63	653	255	353	+45	6.19	3.70	1.28	+1.21	5.68	5.22			diet 5 parathormone 40 units daily
	11–14	28	1058	0	893	+165	653	222	345	−14	6.19	4.15	1.28	+0.76	5.69	5.01			diet 5
	15–18	29	1058	0	849	+209	653	174	386	+93	6.19	3.71	1.29	+1.19	5.28	4.61			diet 5
	19–22	30	1978	0	1669	+309	707	160	499	+48	6.83	3.81	1.44	+1.58	5.14	4.19			diet 5
	23–26	31	1978	0	1885	+93	707	132	578	−3	6.83	4.11	1.52	+1.20	5.59	3.23			diet 5
	27–30	32	1978	0	2180	−202	707	130	687	−110	6.83	4.06	1.87	+0.90	5.35	3.23			diet 5
	31–3 (June)	33	1978	0	1640	+338	707	168	496	+43	6.83	4.02	1.59	+1.22	5.82	3.72	3.42	60	diet 5 Fe Amm. citrate

June	4-7	34	1978	0	1195	+183	707	180	506	+21	6 83	3 95	1 81	+1 07	5 65	4 13						diet 5 Fe Amm citrate
	8	35													6 35	4 60					60	
Oct	10-13	47	415	0	350	+65	962	350	317	+295	9 79	5 25	1 25	+3 29	5 07	5 82	5 54				90	diet A
	14-17	48	115	1	528	−114	962	378	196	+188	9 79	5 93	1 85	+2 01	1 67	7 25	3 87					diet A
	18 21	49	1915	1	1185	+429	962	338	595	+29	9 79	6 73	1 87	+1 19	4 74	7 60	3 27					diet A
	22 25	50	1915	2	1165	+148	962	268	475	+219	9 79	6 18	1 61	+2 00	6 06	5 14	4 82					diet A
	26-29	51	1915	3	1927	−15	962	251	615	+93	9 79	5 88	1 99	+2 00	6 24	5 28	1 16				120	diet A
	30-2 (Nov)	52	1915	5	1389	+521	962	219	172	+241	9 79	6 11	1 50	+2 18	6 52	5 52						diet A
Nov	3 6	53	1919	8	2065	−154	929	185	809	−65	8 90	5 89	2 53	+0 18	6 14	4 90	2 16					diet B Fe Amm citrate
	7-10	54	1919	0	1775	+141	929	170	751	+15	8 90	5 99	2 41	+0 50	6 51	3 71						diet B Fe Amm citrate
	11-14	55	1919	4	1182	+133	929	157	619	+153	8 90	5 75	1 76	+1 39	6 71	3 57	4 13					diet B Fe Amm citrate
	15-18	56	1919	0	1785	+131	929	152	729	+48	8 90	6 21	2 12	+0 57	6 90	3 77						diet B Fe Amm citrate
	19 22	57	1919	2	1940	−23	929	119	798	−18	8 90	5 95	2 12	+0 83	6 91	3 95	5 14				67	diet B Fe Amm citrate
	23 26	58	1919	1	1781	+134	929	187	738	+4	8 96	6 03	2 04	+0 83	6 15	4 13						diet B Fe Amm citrate
	27-30	59	1919	0	1817	+102	929	210	641	+78	8 90	5 99	1 87	+1 01	6 41	4 05	4 10					diet B Fe Amm citrate
Dec	1-4	60	1919	2	1676	+211	929	204	657	+68	8 90	6 91	1 91	+0 98	6 48	4 65	2 92					diet B Fe Amm citrate
	5 8	61	1752	1	1605	+143	642	157	592	−107	7 97	6 03	2 13	−0 19	7 07	4 19	2 61				86	diet C Fe Amm citrate
	9-12	62	1752	4	1533	+215	612	110	578	−16	7 97	5 40	1 98	+0 59	6 32	2 92	2 21					diet C Fe Amm citrate
	13-16	63	1952	4	1640	+308	642	94	490	+58	7 97	1 32	1 71	+1 94	7 06	2 83	3 71					diet C Fe Amm citrate
	17-20	64	1952	3	1682	+267	612	82	505	+55	7 97	4 49	1 90	+0 58	7 11	2 75	2 91					diet C Fe Amm citrate
	21-24	65	1952	2	1637	+313	642	94	579	−31	7 97	1 58	1 91	+1 48	7 18	2 85	3 96				67	diet C Fe Amm citrate
	25-28	66	1952	1	2002	−51	612	86	688	−132	7 97	1 26	2 20	+1 51	7 73	2 84	3 04					diet C Fe Amm citrate
	29-1 (Jan)	67	1952	3	2065	−116	642	112	302	+228	7 69	1 21	1 56	+1 89	7 87	2 77	2 73					diet C
Jan	2-5	68	1952	2	1348	+602	642	116	312	+214	7 69	4 30	1 62	+2 37	7 10	2 78	3 00				16	diet C
	6 9	69	1952	3	1738	+211	642	136	379	+127	7 69	4 70	1 25	+1 71	7 39	3 60	1 86					diet C
	10-13	70	1952	3	1106	+543	612	144	388	+110	7 69	4 83	1 28	+1 38	6 77	3 78	3 52					diet C
	14-17	71	1952	1	1746	+208	612	171	428	+43	7 69	4 18	1 31	+1 90	7 29	3 72	2 69					diet C
	18-21	72	1952	6	1954	−8	642	148	461	+33	7 69	1 78	1 51	+1 10	7 11	3 74	1 82				60	diet C
	22-25	73	1952	1	1872	+79	612	160	419	+33	7 69	4 48	1 50	+1 71	7 10	3 98	0 90					diet C
	26-29	74	1952	4	1349	+599	612	159	311	+169	7 69	1 76	1 17	+1 76	7 08	3 72	1 28				60	diet C A T 10 3 cc daily
	30 2 (Feb)	75	1952	27	1749	+176	642	187	351	+104	7 69	4 95	1 62	+2 12	8 68	1 54	1 56					diet C A T 10 3 cc daily
Feb	3-6	76	1952	19	1239	+664	642	139	381	+122	7 69	1 39	1 39	+1 91	8 32	3 61	0 97					diet C A T 10 3 cc daily
	7-10	77	1952	103	953	+896	612	88	253	+301	7 69	4 32	1 15	+2 22	8 84	3 05	3 53					diet C A T 10 3 cc daily
	11-14	78	1952	163	862	+927	612	77	247	+318	7 69	4 52	1 21	+1 96	9 18	2 92	3 98					diet C A T 10 3 cc daily
	15-18	79	1952	121	1176	+652	642	85	301	+253	7 69	5 08	1 17	+1 11	9 18	3 04	2 16					diet C
	19-22	80	1952	91	1116	+715	642	107	256	+279	7 69	4 66	1 16	+2 17	8 88	3 48	3 66					diet C
	23-26	81	2009	91	1390	+519	626	108	303	+215	7 34	4 65	1 23	+1 46	8 41	3 57	2 31					diet D
	27-1 (Mar)	82	2009	50	1107	+552	626	126	296	+204	7 31	5 10	1 26	+0 98	8 89	3 36	3 31					diet D

* *Phosphatase in Bodansky units per 100 cc*

TABLE 10—*Concluded*

DATE 1939–40	PERIODS 4 DAY	CALCIUM, MG. DAILY				PHOSPHORUS, MG. DAILY				NITROGEN, GM. DAILY				SERUM, MG. %				REMARKS
		Intake	Urine	Stool	Balance	Intake	Urine	Stool	Balance	Intake	Urine	Stool	Balance	Ca	P	Phosphatase*	N.P.N.	
March 2–5	83	2009	47	1420	+542	626	128	330	+168	7.34	4.57	1.27	+1.50	9.28	3.55	4.45		diet D
6–9	84	2009	54	1710	+245	626	139	401	+86	7.34	4.50	1.43	+1.41	8.68	3.32	3.58		diet D
10–13	85	2009	46	1597	+366	626	140	326	+160	7.34	4.25	1.33	+1.76	8.66	3.70	3.00		diet D
14–17	86	2009	13	1630	+366	626	174	335	+117	7.34	4.50	1.38	+1.46	8.18	3.92	4.70		diet D
18–21	87	2009	15	2245	+251	626	161	492	−27	7.34	4.50	1.61	+1.23	7.52	4.39	4.70		diet D
22–25	88	2009	7	1986	+16	626	171	433	+22	7.34	4.63	1.56	+1.15	7.54	3.70	4.63		diet D
26–29	89	2009	1	1580	+428	626	166	356	+104	7.34	5.08	1.16	+1.10	7.61	3.57	4.12	67	diet D
30–2 (Apr.)	90	2009	5	2049	−45	626	152	441	+33	7.34	5.20	1.36	+0.78	8.14	3.69			diet D Vig. 2 cc. daily
April 3–6	91	2009	1	1937	+71	626	175	350	+101	7.34	5.13	1.23	+0.98	7.48	3.75			diet D Vig. 2 cc. daily
7–10	92	2009	5	1712	+292	626	182	356	+88	7.34	4.89	1.13	+1.32	7.43	3.94	3.70		diet D Vig. 2 cc. daily
11–14	93	2009	9	1745	+255	626	206	384	+36	7.34	4.73	1.20	+1.41	6.98	3.92	4.78		diet D Vig. 2 cc. daily
15–18	94	2009	3	1848	+158	626	177	384	+65	7.34	4.85	1.19	+1.30	7.45	3.95	4.90		diet D Vig. 2 cc. daily
19–22	95	2009	2	1736	+271	626	222	355	+49	7.34	4.50	1.16	+1.68	7.78	4.13	4.00		diet D
23–26	96	2009	0	1848	+161	626	158	366	+102	7.34	5.05	1.20	+1.09	7.53	3.77	2.44		diet D
27–30	97	2009	0	2100	−91	626	160	597	−131	7.62	4.98	1.86	+0.78	7.50	3.70	4.05	67	diet D Fe Amm. citrate 6 gm. daily
May 1–4	98	2009	2	1360	+647	626	130	476	+20	7.72	5.10	1.53	+1.09	7.59	3.70	3.15		diet D Fe Amm. citrate 8 gm. daily
5–8	99	2009	4	1496	+509	626	124	514	−12	7.72	4.90	1.60	+1.22	7.48	3.13	4.12		diet D Fe Amm. citrate 8 gm. daily
9–12	100	2009	1	1275	+733	626	132	507	−13	7.82	5.20	1.67	+0.95	7.34	3.20	4.49		diet D Fe Amm. citrate 10 gm. daily
13–16	101	2009	5	1200	+804	626	146	466	+14	7.90	5.30	1.65	+0.87	7.51	3.52	3.89		diet D Fe Amm. citrate 12 gm. daily
17–20	102	2009	8	1220	+781	626	134	464	+28	7.90	5.39	1.73	+0.70	7.76	2.93			diet D Fe Amm. citrate 12 gm. daily
21														7.49	2.96	4.73	75	

TABLE 11

Case 4. K. C. C. Effect of inorganic and dietary phosphorus, vitamin D, A.T.10 and iron on Ca, P and N_2 metabolism

DATE 1940–41	PERIODS 4 DAY	CALCIUM				PHOSPHORUS				NITROGEN				SERUM				REMARKS
		Intake	Urine	Stool	Balance	Intake	Urine	Stool	Balance	Intake	Urine	Stool	Balance	Ca	P	Phosphatase	N.P.N.	
Oct. 19-22	1	271	0	384	−113	618	209	365	+44	6.84	4.23	1.32	+1.29	8.25	4.39	10.99	67	diet 1a
23-26	2	271	0	323	−52	618	250	331	+37	6.84	4.95	1.10	+0.79					diet 1a
27-30	3	271	5	277	−11	618	272	310	+36	6.84	5.92	1.09	−0.17	8.16	4.73	21.58		diet 1a
31-3	4	271	9	296	−34	618	273	472	−73	6.84	6.61	1.26	−1.03	8.06	4.99		97	diet 1a
Nov. 4-7	5	271	1	306	−36	1020	377	451	+192	6.84	6.68	1.08	−0.92	8.04	4.95	22.37		diet 1a Na_2PO_4
8-11	6	271	8	281	−28	1078	487	344	+247	6.84	6.25	0.96	−0.37	6.11	6.17	16.93	100	diet 1a Na_2PO_4
12-15	7	271	4	229	+38	1078	530	218	+330	6.84	5.84	0.68	+0.32	5.58	7.33	15.30	86	diet 1a Na_2PO_4
16-19	8	336	7	350	−21	680	412	470	−202	7.14	5.55	1.09	+0.50	5.38	8.33	18.13	75	diet 1b
20-23	9	336	3	384	−51	680	389	466	−175	7.14	5.52	1.25	+0.37	6.84	6.22	20.38		diet 1c
24-27	10	336	0	485	−149	680	314	463	−97	7.14	4.85	1.64	+0.65	6.94	6.02	22.31		diet 1c
28-1	11	336	0	354	−18	680	346	346	−12	7.14	4.53	1.32	+1.29	7.40	5.28	26.36	86	diet 1c
Dec. 2-5	12	329	2	378	−51	1073	327	652	+94	11.89	6.72	1.47	+3.70	7.73	5.00	27.80		diet 2
6-9	13	329	4	266	+59	1073	334	430	+309	11.89	7.55	1.10	+3.24	7.49	5.81	21.97		diet 2
10-13	14	329	0	522	−193	1073	350	748	−31	11.89	6.98	1.49	+4.42	6.60	6.29	23.02		diet 2
14-17	15	215	1	350	−136	530	326	344	−140	7.20	8.73	0.87	−2.40	6.80	7.11	17.65	150	diet 3a & b
18-21	16	248	0	308	−60	588	349	302	−63	7.29	7.14	0.87	−0.72	6.82	6.23	18.77	120	diet 3c
22-25	17	248	3	399	−151	588	327	404	−143	7.29	6.43	0.90	−0.04	7.10	5.30	17.63	100	diet 3c
26-29	18	1405	0	1328	+77	588	257	476	−145	7.29	5.74	1.22	+0.33	7.54	6.14	18.86		diet 3c
30-2 (Jan.)	19	1405	0	1384	+21	588	224	415	−51	7.29	5.62	1.07	+0.60	7.64	4.35	22.68		diet 3c
Jan. 3-6	20	1451	0	1208	+243	678	205	360	+113	7.80	5.51	0.97	+1.32	8.11	4.39	15.61		diet 4
7-10	21	1451	3	1419	+29	678	187	378	+113	7.80	4.90	1.16	+1.74	8.27	3.76	15.01		diet 4
11-14	22	1151	0	1140	+11	678	171	444	+63	7.80	4.91	1.17	+1.72	8.14	4.02	17.27		diet 4 Vigantol 1 cc. daily
15-18	23	1451	0	1630	−179	678	181	472	+25	7.80	5.11	1.17	+1.52	8.34	3.37	18.81		diet 4 Vigantol 1 cc. daily
19-22	24	1451	4	1308	+139	678	203	422	+53	7.80	5.70	1.20	+0.90	8.00	4.42	13.04		diet 4 Vigantol 1 cc. daily
23-26	25	1556	1	1105	+144	678	180	399	+99	7.80	5.61	1.04	+1.15	7.88	4.46	10.98		diet 4 Vigantol 1 cc. daily
27-30	26	1550	0	1471	+79	678	196	406	+76	7.80	5.82	1.25	+0.73	8.00	4.29			diet 4 Vigantol 1 cc. daily
31-3 (Feb.)	27	1550	0	1180	+370	678	196	316	+136	7.80	5.42	0.98	+1.40	7.72	4.41	20.36		diet 4 Vigantol 1 cc. daily
Feb. 4-7	28	1550	0	1541	+9	678	216	417	+45	7.80	5.57	1.29	+0.94	7.99	4.04	15.36		diet 4 Vigantol 1 cc. daily
8-11	29	1550	2	1350	+198	678	202	360	+116	7.80	5.48	1.25	+1.07	7.92	4.41	17.19		diet 4 A.T.10 3 cc. daily
12-15	30	1550	1	1269	+280	678	201	324	+153	7.80	6.52	1.21	+0.07	8.64	4.75	13.85		diet 4 A.T.10 3 cc. daily
16-19	31	1550	0	926	+624	678	174	271	+233	7.80	7.72	1.38	−1.30	8.39	4.88	12.00		diet 4 A.T.10 3 cc. daily
20-23	32	1550	0	963	+587	678	145	258	+275	7.80	5.06	1.23	+1.51	8.30	4.60	15.94		diet 4 A.T.10 3 cc. daily
24-27	33	1550	0	784	+766	678	110	262	+306	7.80	4.96	1.42	+1.43	8.30	3.85	10.07	67	diet 4 A.T.10 3 cc. daily
28-3 (Mar.)	34	1550	0	735	+815	678	96	223	+359	7.80	6.37	1.23	+0.20	8.55	3.68	17.28		diet 4

TABLE 11—*Concluded*

DATE 1940–41	PERIODS 4 DAY	CALCIUM				PHOSPHORUS				NITROGEN				SERUM				REMARKS
		Intake	Urine	Stool	Balance	Intake	Urine	Stool	Balance	Intake	Urine	Stool	Balance	Ca	P	Phosphatase	N.P.N.	
March 4–7	35	1550	2	754	+794	678	83	262	+333	7.80	6.59	1.37	−0.16	8.44	3.76	16.78		diet 4
8–11	36	1550	5	1066	+479	678	124	367	+187	7.80	6.50	1.49	−0.19	8.24	4.06	15.94		diet 4
12–15	37	1550	7	977	+566	678	114	296	+268	7.80	6.30	1.25	+0.25	8.22	3.82	14.78		diet 4
16–19	38	1550	3	1110	+437	678	132	286	+260	7.80	6.15	1.57	+0.08	8.22	4.06	15.60		diet 4
20–23	39	1550	4	1375	+171	678	145	343	+190	7.80	6.00	1.72	+0.08	7.62	4.29	13.81		diet 4
24–27	40	1550	5	1298	+247	678	128	333	+217	7.80	4.55	1.68	+0.57	8.00	4.71	13.47	75	diet 4
28–31	41	1550	2	1128	+420	678	188	277	+213	7.80	4.71	1.36	+0.73	7.64	4.51	13.43		diet 4
April 1–4	42	1700	3	1227	+470	691	226	328	+137	7.42	4.53	1.55	+1.34	7.40	5.14	15.56		diet 5a
5–8	43	1700	2	1170	+528	691	197	350	+144	7.42	4.44	1.58	+1.40	7.96	4.85	21.25		diet 5a
9–12	44													7.45	5.59	14.89		diet 5a
13–16	45													7.00	6.18	15.56	100	diet 5a
17–20	46	1572	0	1375	+197	624	179	462	−17	6.75	4.85	1.07	+0.83	7.00	5.44	13.94		diet 6a
21–24	47	1677	6	1153	+518	624	142	289	+193	6.75	5.71	0.85	+0.19	7.70	5.28			diet 6a
25–28	48	1677	2	1345	+330	624	155	334	+135	6.75	4.47	1.07	+1.21	7.91	4.63	20.13		diet 6a Vitamin D_2 1,000.000 i.u. oral
29–2 (May)	49	1677	2	911	+764	624	156	208	+260	6.75	4.49	0.92	+1.34	8.47	5.02			diet 6a
May 3–6	50	1677	7	1486	+184	624	180	344	+100	6.75	5.15	0.88	+0.72	7.90	5.31	19.69		diet 6a
7–10	51	1677	0	1249	+428	624	198	284	+142	6.75	4.39	0.85	+1.51	8.12	4.87		83	diet 6a
11–14	52	1677	0	1184	+493	624	145	274	+205	6.75	3.75	1.07	+1.93	8.25	4.61			diet 6a
15–18	53	1677	0	1395	+282	624	137	283	+204	6.75	4.15	1.18	+1.42	7.91	4.59	14.43		diet 6a
19–22	54	1677	0	1482	+195	624	152	378	+94	6.75	3.81	1.41	+1.53	7.94	4.55		67	diet 6a
23–26	55	1671	2	1171	+498	602	134	416	+52	6.61	3.39	1.63	+1.59	8.15	4.60	12.48	59	diet 6a Fe amm. citrate
27–30	56	1671	2	915	+754	602	122	417	+63	6.61	3.42	1.44	+1.75	8.12	4.10			diet 6a Fe amm. citrate
31–3 (June)	57	1671	6	1072	+593	602	118	412	+72	6.61	3.01	1.31	+2.29	7.87	4.30	12.30		diet 6a Fe amm. citrate
June 4–7	58	1671	8	1064	+599	602	98	419	+85	6.61	3.06	1.30	+2.25	7.64	4.39			diet 6a Fe amm. citrate
8–11	59	1671	5	1090	+576	602	109	393	+100	6.61	3.99	1.14	+1.48	8.02	4.06			diet 6a Fe amm. citrate
12	60													8.43	3.94	11.16	60	diet 6a Fe amm. citrate

TABLE 12

Case 5. C. C. C. Effect of dietary phosphorus and vitamin D on Ca, and N_2 metabolism

Date 1940-41	Periods 4-day	Calcium Intake	Calcium Urine	Calcium Stool	Calcium Balance	Phosphorus Intake	Phosphorus Urine	Phosphorus Stool	Phosphorus Balance	Nitrogen Intake	Nitrogen Urine	Nitrogen Stool	Nitrogen Balance	Serum Ca	Serum P	Serum Phosphatase	N.P.N.	Remarks
Nov. 20-23	1	186	6	231	−51	479	123	221	+135	7.63	4.26	0.86	+2.51	7.46	4.70		64	diet 1
24-27	2	186	9	242	−65	479	186	224	+69	7.63	4.41	1.06	+2.16	8.36	3.52	14.73		diet 1
28- 1	3	186	0	286	−100	479	263	273	−57	7.63	4.79	1.24	+1.60					diet 1
Dec. 2- 5	4	1353	2	1126	+225	515	173	306	+36	8.39	5.08	1.25	+2.06	7.84	4.07	20.45		diet 1
6- 9	5	1353	0	1282	+71	515	138	294	+83	8.39	5.21	1.33	+1.85	8.61	3.50	21.20		diet 1
10-13	6	1353	0	1360	−7	515	145	330	+40	8.39	5.63	1.23	+1.48	8.49	3.23	22.96		diet 1
14-17	7	1353	0	1305	+48	515	169	294	+52	8.39	6.01	1.42	+0.96	9.11	3.26	16.19		diet 1 Vigantol 1 cc. daily
18-21	8	1340	0	1151	+189	475	160	224	+91	7.44	4.69	1.18	+1.57	8.64	3.23	19.19		diet 1 Vigantol 1 cc. daily
22-25	9	1356	0	1271	+85	488	163	262	+63	5.74	3.63	1.55	+0.56	8.48	3.89	18.29	32	diet 2 Vigantol 1 cc. daily
26-29	10	1356	0	1295	+61	488	155	273	+60	5.74	3.64	1.45	+0.65	9.05	3.49	18.70		diet 2 Vigantol 1 cc. daily
30- 2 (Jan.)	11	1356	0	1158	+198	488	176	222	+90	5.74	3.91	1.28	+0.55	8.97	3.74	23.96		diet 2
Jan. 3- 6	12	1356	0	1220	+136	488	176	232	+80	5.74	4.16	1.34	+0.24	8.56	3.72	18.07		diet 2
7-10	13	1356	1	1242	+113	488	172	209	+107	5.74	3.57	1.33	+0.84					diet 2
11-14	14	1459	0	1108	+351	907	191	508	+205	8.58	4.69	1.50	+2.39	8.31	3.70	18.52	43	diet 3a
15-18	15	1416	0	846	+570	819	194	388	+237	7.35	5.24	1.22	+0.89	8.38	3.55	17.36	60	diet 3a and b
19-22	16	1270	0	1188	+82	191	170	286	−265	3.41	4.19	1.27	−2.05	8.40	5.36	12.86	86	diet 4a
23-26	17	1421	0	804	+617	415	122	206	+87	4.73	3.48	0.94	+0.31	8.56	3.86	8.72	55	diet 4a
27-30	18	1421	2	1092	+327	415	120	248	+47	4.73	3.46	1.39	−0.12	8.68	3.72		50	diet 4a
31- 3 (Feb.)	19	1421	1	820	+600	415	120	191	+104	4.73	3.96	1.04	−0.27	8.71	3.97	24.77	60	diet 4a
Feb. 4- 7	20	1421	0	965	+456	415	143	178	+94	4.73	3.47	1.12	+0.14	8.53	3.33	21.07	55	diet 4a Vitamin D_2 1,000,000 i.u. oral
8-11	21	1431	0	684	+747	451	84	123	+244	5.49	3.37	1.00	+1.12	8.73	3.48	23.18	50	diet 4b
12-15	22	1431	0	748	+683	451	108	97	+246	5.49	3.78	1.02	+0.69	8.60	3.55	22.79		diet 4b
16-19	23	1431	0	750	+681	451	96	99	+256	5.49	4.21	1.10	+0.18	8.52	3.53	22.31		diet 4b
20-23	24	1431	0	754	+677	451	86	151	+214	5.49	4.00	1.08	+0.41	8.43	3.50	22.34		diet 4b
24-27	25	1431	0	682	+749	451	79	151	+221	5.49	3.95	0.98	+0.56	8.89	3.21	27.95		diet 4b
28- 3 (Mar.)	26	1371	0	866	+505	376	98	154	+124	4.25	4.66	1.04	−1.45	8.60	3.62	24.14		diet 4b
March 4- 7	27	1431	1	675	+755	451	83	167	+201	5.49	4.91	1.03	−0.48	8.80	3.96	20.64		diet 4b
8-11	28	1431	2	940	+489	451	111	240	+100	5.49	4.75	1.33	−0.59	8.90	3.92	17.58		diet 4b
12-15	29	1431	1	885	+545	451	101	220	+130	5.49	4.94	1.37	−0.82	8.72	3.84	15.42		diet 4b

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ANTHROPOMETRY AND ARTHRITIS

I. Differences between Rheumatoid and Degenerative Joint Disease: Males

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INTRODUCTION

It has become increasingly apparent to workers in the field of arthritis that the constitutional approach has much to offer in furthering our knowledge of this disease. Observations on the morphological constitution have been made by clinical observers for several decades (1). In all these instances positive impressions were reported particularly in reference to the differences in body build between rheumatoid arthritics and those persons with degenerative joint disease. These findings, with the exception of Kovacs and Hartung's study (2), were all the result of clinical observations with no actual standard series of measurements of the patients being taken. The work of Kovacs and Hartung revealed marked differences in constitutional morphology between rheumatoid and osteoarthritics but dealt solely with females. In addition, the differences were derived from a limited number of anthropometric measurements and indices. Accordingly, it was deemed desirable by the research committee of the Robert Brigham Hospital to initiate studies on the morphological constitution of arthritics which would be based on (1) a more complete set of anthropometric measurements, indices and observations; (2) a detailed statistical analysis of the data; (3) clear cut diagnosis as to disease type; (4) the inclusion of both sexes.

THE MATERIAL

The material presented in these papers was collected at the Robert Breck Brigham Hospital during the period of September 1938 to January 1940. A

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very large series of anthropometric measurements and observations was taken by the author on a group of almost 400 patients from the wards and the Home Service Clinic. All the subjects were hospital patients at the time of measurement or had been previously hospitalized at the Robert Breck Brigham for their arthritis. The measurements selected and the techniques by which they were taken were those which have been standardized by the Division of Anthropology of Harvard University.

The difficulties involved in measuring arthritic patients with their severe deformities is readily apparent to clinicians. This was circumvented in part by omitting all those measurements of the affected parts of the anatomy of the patients. *No measurements are included in this paper of any parts of the body which according to the medical history or to the eye of the observer had been affected by the arthritic condition of the individual.*

Before grouping the subjects into disease types a review of each case was made by a special committee of the Robert B. Brigham Hospital and final diagnoses were given in the following manner:

"The classification of the patients into arthritic groups by this committee was based on the data contained in the medical records. Where it was feasible to re-examine the patients this was done. The following criteria were used in the grouping process:

1. *Rheumatoid.* Polyarthritis with typical fusiform swelling of at least six months duration. Typical x-ray changes. Elevated sedimentation index.
2. *Degenerative Joint Disease.* Typical x-ray features showing spurring, etc. Relatively low or normal sedimentation index. A minimum of one or two joints acutely involved. Herberden's nodes used for confirmation.
3. *Mixed.* Mixture of rheumatoid and degenerative joint disease criteria.
4. *Strumpel-Marie.* Arthritis of spine. X-ray showing either fusing of sacro-iliac or calcification of spinal ligament or both."

This paper dealing only with the males is limited to a comparative study of the anthropometry of rheumatoid and degenerative joint disease (osteoarthritis) arthritics. It is based on a series of 28 rheumatoids and 20 individuals with degenerative joint disease. These are indeed small samples but they are presented here because their differentiation is so marked it successfully withstands tests of statistical validity. The data on the females are presented in the accompanying paper entitled "Anthropometry and Arthritis. II. Differences Between Rheumatoid and Degenerative Joint Disease: Females."

Additional papers to follow will contain a comparison of the anthropometry of the Strumple-Marie arthritics with the rheumatoid and degenerative joint disease groups, an analysis of the anthropometric status of the mixed arthritics, comparative morphological differences between arthritic types, and a comparison of anthropometry of the arthritics with a control group of non-arthritics.[2]

[2] All the statistical computations were done in the Statistical Laboratory of the Division of Anthropology of Harvard University. Means, ranges, standard deviations and their probable errors, coefficients of variation and their probable errors were computed for all the measurements and indices. For anyone who wishes to examine all the measurements and detailed statistical constants omitted here for lack of space, these are on file and available in the Army Medical Library.

RACE AND NATIONALITY EXTRACTION

In making anthropometric comparisons between the two arthritic groups, rheumatoid and degenerative joint disease, due consideration must be given to the question of their racial make-up. For, if the two groups are found to be markedly divergent in racial composition, then we must assume that at least part of any anthropometric differentiation appearing in the data *may* be attributed to race rather than to the disease itself all other things being equal.

Unfortunately, the size of the two arthritic groups presented here is too small to permit detailed racial analysis. This fact does not entirely preclude the possibility of making some sort of satisfactory racial assessment, if we consider the problem from the point of view of ultimate nationality extraction. Group similarity in nationality extraction usually goes along with group similarity in

TABLE 1

Nationality extraction: Males

	RHEUMATOID				DEGENERATIVE JOINT DISEASE			
	Paternal		Maternal		Paternal		Maternal	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Old American	3	10 7	2	7.1	1	5.0	1	5.0
Irish	7	25 0	10	35 7	7	35.0	7	35.0
English and Scotch	9	32 1	6	21.4	8	40.0	8	40.0
Scandinavian	4	14.3	5	17.9	1	5.0	1	5 0
Italian	2	7 1	2	7.1	2	10.0	2	10.0
Western and Central European mixtures	3	10 7	3	10.7	1	5 0	1	5.0
Totals	28	99 9	28	99.9	20	100 0	20	100.0

racial composition (3). This is particularly true if one is dealing with native born of foreign parentage and foreign born groups. The two arthritic groups considered in this paper are predominantly native born of foreign parents and foreign born.

Table 1 lists the ultimate nationality extractions on both the paternal and maternal sides of the family for the rheumatoid arthritics and those with degenerative joint disease. From these data it may be seen that on the whole the two groups are quite similar in provenience of nationality extraction. In both groups the strongest elements are Irish, on the one hand, and English and Scotch on the other. There are small scatterings of Old American, Scandinavian, Italian, and Western and Central European mixtures. The only differences between the two groups worthy of note are the slight excesses among the degenerative joint disease group of persons with English and Scotch extraction, and the deficiency of individuals of Scandinavian origin. It appears, therefore, that we may safely proceed with the analysis of the anthropometric data with the presumption that the two arthritic series are not markedly divergent in racial composition.

AGE

The frequency distributions for age are given in table 2. As in all other studies those persons with degenerative joint disease are considerably older than the rheumatoid arthritics. The age range for the degenerative joint disease group is from 38 to 78, while that of the rheumatoid arthritics is from 19 to 69. The average age for the rheumatoids is 37.3 years compared to 56.5 years for the degenerative joint disease group, a difference of about 20 years. It is interesting to note that somewhat more than 40 per cent of the rheumatoid series are younger than any of the individuals with degenerative joint disease.

This large age differential between the two arthritic groups raises a serious problem when making anthropometric comparisons inasmuch as there are a number of anthropometric characters in which the age changes are of considerable magnitude. To circumvent this age difficulty I have adopted the following method in handling those morphological features which necessitate age corrections. From the two arthritic groups I have selected those individuals of comparable age. In the case of the rheumatoids I have separated the 12 oldest in the series, and for the degenerative joint disease group the 12 youngest. Thus the twelve oldest rheumatoids range in age from 40 to 65 and have an average age of 46.8 years, and the twelve youngest degenerative joint disease persons range from 38 to 59 with an average age of 49.4 years. The averages for certain anthropometric characters of these two groups have been computed and they are to be used to confirm or negate the directional differences found in the comparisons of the total series.

TABLE 2

Age frequency distributions: Males

AGE	RHEUMATOID	DEGENERATIVE JOINT DISEASE
	number	*number*
19	1	
20–24	3	
25–29	3	
30–34	5	
35–39	4	1
40–44	8	1
45–49	1	4
50–54		3
55–59	1	3
60–64	1	5
65–69	1	
70–74		1
75–78		2
Totals	28	20
Average	37.3	56.5

BODY WEIGHT

In addition to making the simple comparisons between the rheumatoids and degenerative joint disease groups for gross body weight at the time of measurement, it will be necessary to examine the problem of weight loss or gain for our arthritic series. This is an unavoidable procedure owing to the fact that arthritics as a whole frequently undergo large weight changes during the course of the disease.

In the case of the rheumatoid arthritics it is the impression among clinicians that the majority of these patients undergo considerable losses of weight through the disappearance of adipose tissue and muscular atrophy, while this condition is relatively rare in degenerative joint disease sufferers. It should be remembered however, that the degenerative arthritic who complains of symptoms is usually one who is obese to begin with.

Table 3 presents the frequency distributions and averages for gross body weight taken at the time of the anthropological examination. A cursory glance at these figures is sufficient to impress one with the remarkable difference in body weight between the rheumatoids and the degenerative joint disease group. The rheumatoid arthritics are decidedly lighter in body weight with an average of 144 pounds compared with an average of 180 pounds for the degenerative joint disease series. This 36 pound difference between the average weights is a statistically significant difference beyond the error of random sampling. The greater age of the degenerative joint disease group does not appear to be the direct cause of factor in this great weight differential, for, the twelve oldest rheumatoids give an average weight at the time of measurement of 148 pounds against 181 pounds for the twelve youngest degenerative joint disease individuals, a difference between the averages of 33 pounds. It is interesting to note that although the rheumatoid arthritics range all over the weight scale from 102 to 190, the degenerative series have none of their members with body weights under 150 pounds.

For the purpose of estimating gross body weight loss or gain I have selected the "best weights" of each individual as found in the case records of the Robert B. Brigham Hospital. These "best weights" are the weights given by the patients in response to questions by the examining physicians at the time of hospital admission, and are supposed to refer to the patients maximum weight prior to hospitalization. If we examine the frequency distributions and averages of "best weights" in table 3, the rheumatoid arthritics are again unquestionably lighter in weight than the degenerative joint disease group. The average "best weight" for rheumatoids is 154 pounds compared to 186 pounds for the degenerative series, a difference of 32 pounds. And again the age factor can be ignored inasmuch as the "best weight" average is 30 pounds in favor of the youngest degenerative joint disease category in contrast to the oldest rheumatoids.

We may test these results further by comparing weight loss or gain from the "best weight" for each subject individually. The results are to be found in table 4. More than two-thirds of the individuals of both series show their

TABLE 3

Body weight frequency distributions: Males

WEIGHT	RHEUMATOID		DEGENERATIVE JOINT DISEASE	
	When measured	Best weight	When measured	Best weight
lbs.				
100–109	1			
110–119	3			
120–129	3	1		
130–139	4	4		
140–149	7	9		1
150–159	5	3	3	1
160–169	3	3	4	1
170–179		4	2	5
180–189	1	2	4	5
190–199	1		6	3
200–209				1
210–219		1		1
220–229				1
230–239			1	
240–249				
250–259				1
Totals.....	28	27	20	20
Averages.....	144 lbs.	154 lbs.	180 lbs.	186 lbs.

	NUMBER	AVERAGE WEIGHTS	
		When measured	Best weight
Oldest rheumatoids.....	12	148	161
Youngest degenerative joint disease.....	12	181	191

TABLE 4

Comparison between weights when measured and "best weights": Males

	RHEUMATOID		DEGENERATIVE JOINT DISEASE		DIFFERENCE AVERAGE NUMBER OF POUNDS	
	Number	Per cent	Number	Per cent	Rheumatoid	Degenerative joint disease
Weight when measured > "best weight".....	7	25.9	7	35.0	11.6	7.1
Weight when measured = "best weight".....	0	0	2	10.0		
Weight when measured < "best weight".....	20	74.1	11	55.0	17.2	17.5
Totals.....	27	100.0	20	100.0		

weights when measured to be less than their "best weights." This occurs somewhat more often among the rheumatoids than in the degenerative group (74 per cent to 55 per cent). The remainder of the individuals, whose weights

when measured were equal to or greater than their "best weights", are more highly represented among the degenerative group than among the rheumatoids (45 per cent to 26 per cent). However, it is highly significant to note that when the weights at the time of measurement are less than the "best weights", the average weight loss for the rheumatoids is approximately the same as for the degenerative series (17.2 pounds to 17.5 pounds respectively).

To summarize this problem of weight it would appear from the data of the Robert B. Brigham Hospital that rheumatoid arthritics average around 30 pounds lighter in gross body weight than individuals with degenerative joint disease. This differential is not due to the ravages of the disease but represents the constitutional difference in body weight between rheumatoids and degenerative joint disease persons. Rheumatoid arthritics when showing weight losses have no significantly greater weight losses than the degenerative joint disease individuals, and interestingly enough show larger average weight gains when their weight at the time of the disease is better than their "best weights".

The above evidence would indicate that it would be safe to use those anthropometric measurements for comparison of our arthritic series in which body weight is a factor. In both groups the overall weight change is in the same direction and approximately of the same magnitude.

GROSS BODY MEASUREMENTS AND PROPORTIONS

Table 5 compares the rheumatoid arthritics and those persons with degenerative joint disease with respect to certain gross anthropometric measurements and proportions. Only those measures which present significant mean differences between the two groups are listed with their statistical constants. For each statistically significant measure the table gives the number of individuals in each group, the range, the mean and its probable error, the difference between the means, the probable error of this difference, and the critical ratio.[3] It may be seen that statistically significant differences between the arthritic groups appear in the case of the maximum arm span and the span/stature index. Rheumatoid arthritics have shorter arm spans than the degenerative joint disease group both in absolute dimensions and relative to the total body height. This difference in mean arm span of 3.73 centimeters is a large difference, and its statistical significance is all the more remarkable considering the small number of rheumatoids available in the series whose arms, wrists and hands were not affected by the disease, thus giving a reliable measurement.

In other gross body measurements and proportions such as stature, sitting height, trunk height, and their concomitant indices, no appreciable differences between the two arthritic groups could be ascertained.

[3] The critical ratio is simply the difference between the means divided by the probable error of the difference. A critical ratio of three or more is considered here to be statistically significant, for such a difference could only occur as a result of a sampling error in four cases out of one hundred. The larger the critical ratio the smaller the possibility that such a difference could have arisen as a result of chance sampling. Thus a critical ratio of four signifies that such a difference between the means could only occur as a result of chance sampling in seven cases out of one thousand trials.

larger sternal lengths than the degenerative group but is due wholly to the narrower chest breadths of the rheumatoids.

TABLE 7

Upper torso measurements and indices: Males

	NUMBER	RANGE	MEAN P.E.	COMPARISON BETWEEN MEANS		
				Diff.	P.E.	Critical ratio
Measures showing significant mean differences:						
Biacromial diameter:						
Rheumatoid	28	33.5– 42.4	37.77 ± .26	−1.05	± .37	2.84
Degenerative	20	36.0– 43.4	38.82 ± .26			
Chest breadth:						
Rheumatoid	28	23.5– 31.9	27.18 ± .24	−2.60	± .35	7.43
Degenerative	20	25.0– 32.4	29.78 ± .25			
Chest depth:						
Rheumatoid	28	16.8– 26.6	21.00 ± .26	−2.35	± .34	6.91
Degenerative	20	20.4– 26.0	23.35 ± .22			
Chest circumference:						
Rheumatoid	28	77.0–108.9	88.59 ± .83	−10.26	± 1.17	8.77
Degenerative	20	88.0–110.9	98.85 ± .82			
Interpapillary diameter:						
Rheumatoid	28	15.9– 26.0	21.08 ± .25	−2.53	± .44	5.75
Degenerative	19	19.2– 29.3	23.61 ± .36			
Intercostal angle:						
Rheumatoid	28	6 – 71	37.22 ± 1.69	−13.06	± 2.29	5.70
Degenerative	19	32 – 75	50.28 ± 1.54			
Chest breadth/stature index:						
Rheumatoid	20	13.6– 18.7	15.57 ± .18	−1.80	± .25	7.20
Degenerative	17	14.6– 18.7	17.37 ± .18			
Chest circumference/stature index:						
Rheumatoid	20	45.0– 56.9	50.62 ± .48	−6.74	± .80	8.42
Degenerative	17	48.0– 64.1	57.36 ± .64			
Sternal/chest breadth index:						
Rheumatoid	28	47.6– 76.2	61.15 ± .73	6.77	± 1.27	5.33
Degenerative	19	42.7– 74.1	54.38 ± 1.04			
Biacromial/trunk height index:						
Rheumatoid	26	57.0– 68.4	62.68 ± .42	−2.99	± .91	3.29
Degenerative	17	59.5– 77.9	65.67 ± .81			
Measures showing no significant differences:						
Chest length, sternal length, biacromial/stature index, chest index						

How great a difference exists between the two groups with respect to the 'onship of the circumference to stature may best be seen in table 10 the frequen "utions of this proportion are listed. Only about

one-half of the distributions of the two groups overlap. None of the rheumatoids has a chest circumference/stature index over 57 0, while 11 out of 17 individuals with degenerative joint disease or 65 per cent of the group have indices over this figure.

TABLE 8

Age comparisons for upper torso measurements and indices Males

	NUMBER	MEAN
Measures showing significant mean differences in original comparisons		
Biacromial diameter		
Old rheumatoids	12	37 3
Young degeneratives	12	38 9
Chest breadth		
Old rheumatoids	12	27 6
Young degeneratives	12	30 4
Chest depth		
Old rheumatoids	12	22 4
Young degeneratives	12	23 2
Chest circumference		
Old rheumatoids	12	91 5
Young degeneratives	12	101 6
Interpapillary diameter		
Old rheumatoids	12	21 8
Young degeneratives	12	23 7
Intercostal angle		
Old rheumatoids	12	42 0
Young degeneratives	12	48 0
Chest breadth/stature index		
Old rheumatoids	8	15 9
Young degeneratives	11	17 1
Chest circumference/stature		
Old rheumatoids	8	52 2
Young degeneratives	11	56 9
Sternal/chest breadth		
Old rheumatoids	12	61 2
Young degeneratives	12	53 4
Biacromial/trunk height		
Old rheumatoids	12	62 2
Young degeneratives	12	61 8

When age corrections are made for those upper torso measurements and indices which show significant mean differences between the rheumatoids and the degenerative series we find that the directions of the differences are substantiated in all cases with the exception of the biacromial/trunk height index (table 8). The "old rheumatoids" contrasted with the "young degeneratives" for the relationship of shoulder breadth to trunk height are not relatively narrower in the shoulders as was found in the total series As a matter of fact the figures indicate that they may be slightly broader-shouldered relative to

TABLE 9

Frequency distribution for intercostal angle: Males

INTERCOSTAL ANGLE	RHEUMATOID	DEGENERATIVE JOINT DISEASE
	number	*number*
7–9	1	
10–14		
15–19	1	
20–24	2	
25–29	2	
30–34	7	1
35–39	4	1
40–44	4	4
45–49	3	4
50–54	1	4
55–59	1	1
60–64	1	3
65–69		
70–74	1	
75–79		1
Totals.....	28	19

TABLE 10

Frequency distribution for chest circumference/stature index: Males

CHEST CIRCUMFERENCE/STATURE	RHEUMATOID	DEGENERATIVE JOINT DISEASE
	number	*number*
45.0–46.1	3	
46.2–47.3	1	
47.4–48.5	2	1
48.6–49.7		
49.8–50.9	4	
51.0–52.1	3	1
52.2–53.3	3	1
53.4–54.5	2	1
54.6–55.7	1	1
55.8–56.9	1	1
57.0–58.1		2
58.2–59.3		4
59.4–60.5		2
60.6–61.7		2
61.8–62.9		
63.0–64.1		1
Totals.....	20	17

their trunk heights than the degenerative group. Trunk height tends to diminish from maturity onwards. This makes the biacromial/trunk height increase with age, and since the degenerative group is considerably older than the

rheumatoid series the significant difference between them may be entirely ascribed to their age differential.

LOWER TORSO MEASUREMENTS AND PROPORTIONS

Table 11 gives the statistical constants for those measures of the lower torso showing significant mean differences between the rheumatoids and the degenera-

TABLE 11

Lower torso measurements and indices: Males

	NUMBER	RANGE	MEAN P.E.	COMPARISON BETWEEN MEANS		
				Diff	P.E.	Critical ratio
Measures showing significant mean differences:						
Bi-iliac diameter:						
Rheumatoid	28	26 1–31 7	28.92 ± 18	−1 54 ±	28	5 50
Degenerative	20	28 2–34 1	30 46 ± 21			
Umbilical circumference						
Rheumatoid	28	62 0–102 4	74 97 ± 1 17	−15 21 ±	1 68	9 05
Degenerative	19	77 0–108 4	90.18 ± 1.21			
Bi-iliac/stature index.						
Rheumatoid	20	14 6–18 5	16 76 ± 14	−1 04 ±	.20	5.20
Degenerative	17	15 8–19.0	17 80 ± 14			
Bi-iliac/chest breadth index:						
Rheumatoid	28	93 0–123 0	106 56 ± .84	4.06 ±	1 28	3 17
Degenerative	20	93 0–123 9	102 50 ± .96			
Bi-iliac/trunk height index.						
Rheumatoid	26	42 5–52 0	47.85 ± .20	−3.05 ±	.50	6 10
Degenerative	17	46 5–55.0	50 90 ± .41			
Measures showing no significant differences:						
Total abdominal length, upper abdominal length, lower abdominal length, upper abdominal/torsal index, lower abdominal/torsal index, sternal/abdominal index, upper abdominal/lower abdominal index, bi-iliac/biacromial index						

tive joint disease group. From these data the rheumatoid arthritics appear to be somewhat narrower in the breadth across the hips (bi-iliac diameter), very markedly smaller in abdominal circumference, a little narrower in the hips relative to stature, markedly broader in the hips relative to the chest breadth, and narrower in the hips relative to the trunk height. The large weight differential between the rheumatoids and the degenerative joint disease group

probably accounts for the greater part of the difference in abdominal circumference and possibly of the bi-iliac diameter.

A comparison between the "old rheumatoids" and "young degeneratives" for these same measurements and indices reveals similar directional differences in every instance. However, the extent of the difference is very much reduced in the case of the bi-iliac/stature index and the bi-iliac/trunk height index which may indicate that in these proportions we are dealing with an age factor.

It is noteworthy that no statistically significant differences between the two arthritic groups were found for any of the abdominal segments and proportions.

TABLE 12

Age comparisons for lower torso measurements and indices: Males

	NUMBER	MEAN
Measures showing significant mean differences in original comparisons:		
Bi-iliac diameter:		
Old rheumatoids	12	29.5
Young degeneratives	12	30.5
Umbilical circumference:		
Old rheumatoids	12	78.7
Young degeneratives	12	89.6
Bi-iliac/stature index:		
Old rheumatoids	8	17.4
Young degeneratives	11	17.6
Bi-iliac/chest breadth index:		
Old rheumatoids	12	107.4
Young degeneratives	12	103.0
Bi-iliac/trunk height index:		
Old rheumatoids	12	49.3
Young degeneratives	12	50.9

UPPER EXTREMITY MEASUREMENTS AND PROPORTIONS

The rheumatoid arthritics and the degenerative joint disease group are quite dissimilar in the dimensions and proportion of the upper extremities. The data in table 13 show the rheumatoids to be considerably smaller than the degenerative group in distal humeral breadth (breadth across the epicondyles), much narrower in breadth of wrists, markedly smaller in both hand length and hand breadth, and slightly shorter in the length of the forearm. The hand index (hand breadth divided by the hand length) is significantly lower among the rheumatoids indicating the possession by the latter of relatively longer and narrower hands. And finally the forearm relative to the length of the upper arm is considerably shorter in the rheumatoids than in the degenerative joint disease group.

Table 14 shows that the above differences cannot be due to the age differential between the two series, although it appears that in two or three instances the size of the differences is greatly reduced.

LOWER EXTREMITY MEASUREMENTS AND PROPORTIONS

There is very little difference between the rheumatoids and the degenerative joint disease group in the absolute dimensions of the lower extremities with the exception of the calf circumference. Table 15 shows that the rheumatoids are significantly smaller in calf circumference to the extent of slightly more than 4 centimeters. It is certain that at least a part of this difference is due to the fact

TABLE 13

Upper extremities measurements and indices: Males

	NUMBER	RANGE	MEAN P.E.	COMPARISON BETWEEN MEAN	
				Diff. P.E.	Critical ratio
Measures showing significant mean differences:					
Distal humeral breadth:					
Rheumatoid	27	60.0– 78.0	69.70 ± .50	−3.05 ± .74	4.12
Degenerative	20	68.0– 80.0	72.75 ± .54		
Wrist breadth:					
Rheumatoid	21	50.0– 64.0	57.29 ± .49	−3.36 ± .77	4.36
Degenerative	20	54.0– 69.0	60.65 ± .59		
Hand length:					
Rheumatoid	20	173.0–204.0	188.25 ± 1.22	−5.95 ± 1.70	3.50
Degenerative	20	176.0–209.0	194.20 ± 1.19		
Hand breadth:					
Rheumatoid	19	70.0– 91.0	81.79 ± .76	−5.51 ± 1.05	5.25
Degenerative	20	80.0– 99.0	87.30 ± .72		
Forearm length:					
Rheumatoid	20	21.9– 26.6	24.19 ± .20	−1.11 ± .34	3.26
Degenerative	18	21.3– 29.0	25.30 ± .28		
Hand index:					
Rheumatoid	18	40.5– 47.0	43.57 ± .33	−1.45 ± .48	3.02
Degenerative	20	40.5– 49.7	45.02 ± .35		
Forearm/upper arm index:					
Rheumatoid	20	68.4– 84.5	75.82 ± .59	−3.58 ± .89	4.02
Degenerative	18	67.5– 88.1	79.40 ± .67		
Measures showing no significant differences:					
Arm length, upper arm length					

that the rheumatoids are so much lighter in body weight than the degenerative group.

Where large and significant differences are to be found they appear in the relative proportions of the lower extremity dimensions to other parts of the body. Thus significant differences exist for the calf circumference/biacromial index, the distal femoral breadth/chest circumference index, and the ankle breadth/chest circumference index. Other indices of the same sort could be

computed which would show significant differentiation between the two groups, but the above are sufficient to illustrate the relative proportions of the lower extremities to other parts of the body.

The rheumatoid arthritics have considerably smaller calf circumference relative to shoulder breadth (calf circumference/biacromial) than the degenerative joint disease group. Again the weight differential between the two arthritic groups must be considered as an important factor influencing the size and possibly even the direction of the difference. There is virtually no distinction

TABLE 14

Age comparisons for upper extremities measurements and indices: Males

	NUMBER	MEAN
Measures showing significant mean differences in original comparisons:		
Distal humeral breadth:		
Old rheumatoids	12	70.6
Young degeneratives	12	72.2
Wrist breadth:		
Old rheumatoids	10	57.8
Young degeneratives	12	60.8
Hand length:		
Old rheumatoids	8	190.4
Young degeneratives	12	196.9
Hand breadth:		
Old rheumatoids	8	83.8
Young degeneratives	12	87.2
Forearm length:		
Old rheumatoids	8	24.2
Young degeneratives	11	25.9
Hand index:		
Old rheumatoids	6	44.0
Young degeneratives	12	44.3
Forearm/upper arm index:		
Old rheumatoids	9	79.0
Young degeneratives	11	80.4

between the two groups in the relationship of the calf circumference to the chest circumference at rest.

The most important differences between the rheumatoids and the degenerative joint disease group lie in the relative proportions of the epicondylar breadth of the femur to the chest circumference (distal femoral/chest circumference), and in the relative proportions of the width of the distal end of the tibia to the chest circumference, (ankle breadth/chest circumference). Both the femoral epicondylar breadth and the distal tibial breadth are considerably larger in the rheumatoids than in the degenerative group when these skeletal dimensions are related to the size of the chest. The differences shown by these indices are due principally to the larger chest size of the degenerative joint disease group rather than to any distinctions in robusticity of the femur or tibia. It should be noted

TABLE 15

Lower extremities measurements and indices Males

	NUMBER	RANGE	MEAN P E	COMPARISON BETWEEN MEANS	
				Diff P E	Critical ratio
Measures showing significant mean differences					
Calf circumference					
Rheumatoid	27	25 0– 34 9	30 94 ± 31	−4 04 ± 48	8 42
Degenerative	20	30 0– 39 9	34 98 ± 38		
Calf circumference / biacromial diameter index					
Rheumatoid	27	67 0– 98 0	81 72 ± 88	−8 38 ± 1 30	6 45
Degenerative	20	75 0–102 0	90 10 ± 96		
Distal femoral breadth/chest circumference (rest)					
Rheumatoid	22	98 0–123 0	107 50 ± 96	6 25 ± 1 39	4 50
Degenerative	16	90 0–109 0	100 25 ± 1 01		
Ankle breadth/chest circumference (rest)					
Rheumatoid	24	70 0– 99 0	80 08 ± 94	8 52 ± 1 18	7 22
Degenerative	18	63 0– 78 0	71 56 ± 72		
Measures showing no significant differences					
Distal femoral breadth, ankle breadth, tibiale sphenion length, lower extremity length, tibiale/stature, intermembral index, calf circumference/chest circumference (rest)					

TABLE 16

Age comparisons for lower extremities measurements and indices Males

	NUMBER	MEAN
Measures showing significant mean differences in original comparisons		
Calf circumference		
C[illegible]	12	30 7
Y	12	35 1
Calf circumference/biacromial diameter		
Old rheumatoids	12	82 3
Young degeneratives	12	90 2
Distal femoral/chest circumference (rest)		
Old rheumatoids	11	105 0
Young degeneratives	11	98 8
Ankle breadth/chest circumference (rest)		
Old rheumatoids	10	75 9
Young degeneratives	11	70 5

that here we are dealing with very large differences, 6.25 index units in the case of the distal femoral/chest circumference index, and 8.52 units for the ankle breadth/chest circumference relationship. In the case of the latter index the complete range of the degenerative joint disease group (63–78) only overlaps about 50 per cent of the rheumatoid range (70–99), while for the distal femoral/chest circumference index the complete range of the degenerative series (90–109) encompasses but 60 per cent of the rheumatoid range (98–123).

The age comparisons in table 16 support the direction of the differences observed for all the significant measures of the lower extremities.

To summarize all the data on the lower extremities, it would appear first, that the degenerative joint disease group surpasses the rheumatoids in absolute size of muscular circumferences; second, that no significant differences exist between the two groups with respect to skeletal robusticity; third, that the degenerative joint disease group have much larger upper torso dimensions than the rheumatoids relative to the breadth of the knee and ankle joints; and fourth, if further statistical computations were made the degenerative group would also present smaller knee and ankle joints relative to the size of the head, face, upper extremities, and lower torso regions, all indicating a marked tapering in the lower extremities.

HEAD, FACE, AND NECK MEASUREMENTS AND PROPORTIONS

The outstanding feature in this area of the body is the continuation of the general superiority of the lateral dimensions of the degenerative group over those of the rheumatoid arthritics. Although this may be seen in the measurements of the cranial vault, the significant differences between the means of the two arthritic groups appear only in the measurements and proportions of the face. Thus we find in table 17 that the rheumatoid arthritics are significantly narrower than the degenerative joint disease group in facial breadth (bizygomatic diameter), in the breadth of the hinder ends of the lower jaw (bigonial diameter), and in the breadth of the nose. They also have narrower foreheads relative to the width of the hinder ends of the lower jaws (fronto-gonial index), narrower facial breadth relative to the hinder ends of the lower jaw (zygo-gonial index), but they exhibit a wider set of the eyes relative to the facial width (ocular zygomatic index).

The circumferences of the head and neck are significantly smaller in the rheumatoids. The length of the external ear is considerably shorter in the rheumatoid arthritics and the ear index being significantly higher indicates the possession of relatively broader and shorter ears.

The age comparisons in table 18 confirm all the above differentiations between the rheumatoids and the degenerative arthritics with the exception of ear length. This difference between the two arthritic groups is clearly the result of the age factor making for longer ears in the older degenerative joint disease individuals. It should also be pointed out that the size of the difference in ear index is considerably reduced by the comparison of the "old rheumatoids" and "young degeneratives".

TABLE 17

Head, face, neck measurements and indices Males

	NUMBER	RANGE	MEAN P.E	COMPARISON BETWEEN MEANS Diff P.E	Critical ratio
Measures showing significant mean differences					
Head circumference					
Rheumatoid	27	53 3- 59 5	55 91 ± 18	−1 13 ± 28	4 04
Degenerative	20	54 2- 59 8	57 04 ± 21		
Bizygomatic diameter					
Rheumatoid	28	130 0-158 0	139 50 ± 72	−4 65 ± 96	4 84
Degenerative	20	132 0-151 0	144 15 ± 63		
Bigonial					
Rheumatoid	28	96 0-117 0	105 54 ± 65	−6 46 ± 92	7 02
Degenerative	20	105 0-124 0	112 00 ± 65		
Nose breadth					
Rheumatoid	28	31 0- 40 0	34 89 ± 31	−2 61 ± 54	4 83
Degenerative	20	31 0- 44 0	37 50 ± 44		
Ear length					
Rheumatoid	28	54 0- 76 0	64 64 ± 67	−3 81 ± 98	3 89
Degenerative	20	60 0- 80 0	68 45 ± 72		
Neck circumference					
Rheumatoid	28	30 0- 38 9	35 32 ± 26	−2 19 ± 35	6 26
Degenerative	20	33 6- 41 0	37 51 ± 24		
Fronto/gonial index					
Rheumatoid	28	91 0-110 9	100 81 ± 58	−3 54 ± 1 10	3 22
Degenerative	20	94 0-117 9	104 35 ± 94		
Zygo/gonial index					
Rheumatoid	28	68 6- 83 2	75 93 ± 38	−1 83 ± 59	3 10
Degenerative	20	73 5- 86 7	77 76 ± 45		
Ocular/zygomatic					
Rheumatoid	28	59 5- 69 4	64 32 ± 29	2 14 ± 41	5 22
Degenerative	20	56 0- 64 9	62 18 ± 29		
Ear index					
Rheumatoid	28	49 7- 63 6	57 05 ± 47	2 78 ± 80	3 48
Degenerative	20	46 2- 62 2	54 27 ± 65		
Measures showing no significant differences					
Head length, head breadth, head height, minimum frontal diameter, total face height, upper face height, lower face height, nose height, biocular diameter, interocular diameter, ear breadth, breadth/length cranial index, length/height cranial index, breadth/height cranial index, fronto/parietal index, cephalo / facial index, zygo/ frontal index, total facial index, upper facial index, nasal index, head and neck height, head shoulder height					

SOMATOTYPES

In addition to the detailed anthropometric measurements each subject was given a general body build classification called a somatotype. The somatotype is a body build classification based on a modified Kretschmerian system whereby each individual is rated for three bodily components, pyknic, somatic, and

TABLE 18

Age comparisons for head, face, neck measurements and indices: Males

	NUMBER	MEAN
Measures showing significant mean differences in original comparisons:		
Bizygomatic diameter:		
Old rheumatoids	12	140.8
Young degeneratives	12	144.7
Bigonial:		
Old rheumatoids	12	107.9
Young degeneratives	12	111.6
Nose breadth:		
Old rheumatoids	12	35.4
Young degeneratives	12	37.8
Ear length:		
Old rheumatoids	12	67.0
Young degeneratives	12	67.2
Fronto/gonial index:		
Old rheumatoids	12	102.4
Young degeneratives	12	103.4
Zygo/gonial index:		
Old rheumatoids	12	76.7
Young degeneratives	12	77.1
Ocular/zygomatic:		
Old rheumatoids	12	64.5
Young degeneratives	12	62.9
Ear index:		
Old rheumatoids	12	56.8
Young degeneratives	12	55.0
Neck circumference:		
Old rheumatoids	12	35.4
Young degeneratives	12	37.4
Head circumference:		
Old rheumatoids	12	55.9
Young degeneratives	12	57.4

leptic (5). The pyknic component is the element of softness and roundness. The somatic component refers to the amount of bone and muscle development, while the leptic component is the element of linearity and fragility. Each of these components is rated on a scale from one to seven. The low figure represents that component in its minimum development and the high figure its maximum development.

The considerable variation in the nutritive conditions of the arthritic subjects,

weight losses, weight gains, muscular atrophy, et cetera, made it extremely difficult to form accurate somatotype rating judgements. Accordingly, they are presented here with some reservation as to their reliability. The reader may judge for himself the extent of their accuracy from the manner in which they confirm or negate the trends of the detailed anthropometry.

Table 19 compares the rheumatoid arthritics and the degenerative joint disease group for somatotype ratings in each of the three components, pyknic, somatic, and leptic. From these data it may be seen that the rheumatoid arthritics are somewhat lower than the degenerative joint disease group in the pyknic component. The average rating for the rheumatoids is 2.3 against 2.9 for the degenerative series. This implies that the rheumatoids have less of the element of softness and roundness in their bodily make-up. However, the less favorable nutritional status of the rheumatoids may account for part

TABLE 19

Somatotypes: Males

RATING	PYKNIC COMPONENT		SOMATIC COMPONENT		LEPTIC COMPONENT	
	Rheumatoids	Degeneratives	Rheumatoids	Degeneratives	Rheumatoids	Degeneratives
	number	*number*	*number*	*number*	*number*	*number*
1	4		1			3
2	15	8	4	1	4	11
3	7	8	8	1	10	3
4	2	3	9	4	5	1
5		1	5	8	7	2
6			1	5	2	
7				1		
Totals	28	20	28	20	28	20
Averages .	2.3	2.9	3.6	4.9	3.8	2.4

of this difference. There is an exceedingly large difference between the two arthritic groups in the somatic component. In this instance the degenerative joint disease group is far greater in amount of bone and muscle development than the rheumatoids. There is little question that this is a real and significant difference. The average somatic rating for the degenerative group is 4.9 compared to only 3.6 for the rheumatoids. The leptic component reveals another very large difference between the two series with the rheumatoids more strongly on the linear side in body build. The average leptic rating for the rheumatoids is 3.8 against 2.4 for the degenerative group. On the basis then of the averages of the somatotypes, the degenerative arthritics may be described as being medium in the pyknic component, very high in the somatic component, and distinctly submedium in the leptic. The rheumatoid arthritics are submedium in the pyknic element, and medium in the somatic and leptic components.

When the complete somatotype rating for each subject is considered as a single unit, it is found that the members of both arthritic series group themselves into a number of rather clear cut types. The rheumatoid arthritics consist in the main of two types of individuals. The first type is low in the pyknic component, moderate to low in the somatic component, and high in the leptic element. The second type is moderate to low in the pyknic, moderate to high in the somatic, and only moderate in the leptic element. There is also a small residuum of individuals who are moderate to high in the pyknic, high in the somatic, and low in the leptic component. Among the degenerative joint disease group the great majority of individuals are moderate to low in the pyknic component, very high in the somatic, and low in the leptic element. In addition there is a small group who are strong in the pyknic element, high in the somatic, and very low in the leptic component. And only an occasional individual appears among the degenerative joint disease group with a low pyknic rating, low somatic, and very high leptic components.

None of the arthritic groups seem to be especially high in the female component, but with respect to dysplasia or asymmetries of parts of the body considerable difference was observed between the two groups. The degenerative joint disease groups presented a much higher proportion of individuals who were dysplastic than the rheumatoids. Among the degenerative joint disease group this took the form principally of a tapering in the lower extremities, while in the rheumatoid series the dysplasias were seen in the weakness of the chest and upper extremities.

DISCUSSION

After examination of this large number of anthropometric measurements and proportions, it is perfectly clear that the rheumatoid arthritics are markedly different in bodily physique from those persons with degenerative joint disease. These differences persist even after allowances have been made for the age discrepancies between the two groups and the effect of the disease upon various parts of the body.

From the profusion of detailed distinctions between the two arthritic series several of the outstanding differentiations of a more comprehensive nature merit further discussion. To begin with the clinical impressions of many observers[4] who referred to the rheumatoid arthritics as being the slender, asthenic, viscerptotic type, are not borne out by the results of this study. It is true, of course, that such a type is to be found among the rheumatoid arthritics but it is only one element among several others. The type which exhibits a moderate amount of linearity and is moderate to high in the somatic bone-muscle component is of equal importance with the strongly linear type. Where the clinical observers are correct is in asserting that the rheumatoid arthritics are *more linear* than those individuals with degenerative joint disease. For the main point is not the linearity of the rheumatoids but the strong laterality, stocky build, big-

[4] See Introduction, page 000.

boned, big-muscled, and high pyknic character of the degeneratives. The emphasis then should be placed on the body build of the degenerative joint disease group, and this is substantiated by the fact that this group exhibits greater anthropological distinctiveness and homogeneity than the rheumatoids.

Another point of particular interest is the marked tapering of the lower extremities among the degenerative joint disease group. When compared with the rheumatoids we find that the degeneratives have absolutely and relatively larger bodily dimensions above the hips for the size of the knee and ankle joints. Since the body mass which the knee and ankle joints must support is far greater among the degenerative joint disease group than among the rheumatoids, it is interesting to speculate whether this relative lack of robusticity of the knee and ankle joints of the degenerative joint disease group is related in any way to the frequency of the appearance of the disease in the lower extremities of the body. The writer is not unaware of the fact that all or almost all human beings have varying degrees of joint changes of a degenerative nature by the time they reach the fifth decade of life. One might therefore expect all bodily types to be represented in a group of people with degenerative joint disease. That this does not occur, leads one to suspect that the strongly lateral, big-boned, big-muscled, and highly pyknic type of individual is the type which shows more degenerative arthritic symptoms, has more disability, and eventually requires hospitalization.

It may be suggested by some readers that this degenerative series by virtue of its characteristic physique may simply represent a special group of cases of functional traumatization of the weight bearing joints accompanied by symptoms severe enough to eventually require hospitalization. In other words, this strongly lateral, pyknic, big-boned, big-muscled, top heavy, tapering type of individual because of his added weight and possibly increased activity may have traumatized pre-existing hypertrophic changes in the weight bearing joints so that they have become symptomatic and that therefore what we are dealing with here cannot be considered as representing the situation for the complete syndrome of hypertrophic arthritis. If this were true, we should then expect that our series of hypertrophic arthritics should present a history of symptoms principally limited to the weight bearing joints, particularly in the knees, with the virtual exclusion of symptoms and hypertrophic changes in other parts of the body. Accordingly our series has been reviewed for location of hypertrophic changes and the results of this survey are to be seen in table 20.

From the data in this table it is clearly apparent that extensive hypertrophic changes are manifest very frequently in other parts of the body apart from the weight bearing joints acutely involved. These are not exclusively knee cases. The hands reveal hypertrophic symptoms and changes virtually as often as seen in the knees. Feet, hips, back, neck, lumbar spine, wrists, shoulders, et cetera are also included.

We may safely conclude, therefore, that degenerative-hypertrophic changes in our hospital cases are more common in the physical type represented by our

degenerative arthritic series, this being true apart from possible factors of increased trauma due to marked weight and activity.

The results of this paper indicate that further investigations of the foregoing anthropological nature should be undertaken in connection with the study of arthritis. A modest beginning has been made. The results communicated

TABLE 20

Location of hypertrophic changes: Males

CASE	ADMISSION COMPLAINTS	ADDITIONAL SITES REVEALED BY PHYSICAL EXAMINATION	ADDITIONAL SITES REVEALED BY X-RAY*
Hosp. No. 1302	Knees		Hands, feet, hips, lumbo-sacral
Hosp. No. 1638	Knees		Hands, wrists
Hosp. No. 1500	Knees	Hands, hips	Hands, feet, lumbar, spine
Hosp. No. 1777	Hips, hands, knees		Sacro-iliac, lumbo-sacral
Hosp. No. 1381	Lower back, hip, shoulders	Knees	Hands, wrists, feet
Hosp. No. 1198	Lower back, hips	Knees, ankles	Hands, spine, feet
Hosp. No. 1669	Lumbar spine	Neck	
Hosp. No. 1893	Hip		Lumbo-sacral
Hosp. No. 1732	Lower back	Hands, lumbar spine, sacro-iliac	Wrists, knees
Hosp. No. 1614	Lower back, leg	Hip, knees	
Hosp. No. 1069	Shoulder, back, wrists, hands, feet		Neck
Hosp. No. 1770	Lower back		Hands, wrists, feet
Hosp. No. 1408	Knees, feet, hips, wrists, shoulders		Sacro-iliac, lumbar region
Hosp. No. 1632	Neck	Ankles, lumbo-sacral	
Hosp. No. 1537	Leg, knee, hips	Sacro-iliac	Lumbo-sacral
Hosp. No. 1300	Arm, hip, leg, shoulder, hands	Knees, neck, lumbo-sacral	Feet
Hosp. No. 1587	Lower back	Knees, hip	Hands, feet, lumbo-sacral
Hosp. No. 1662	Hips, ankles	Knees, hands	
Hosp. No. 1670	Shoulder, knees, hands, lumbar spine	Neck	
Hosp. No. 1657	Hip	Shoulders, lumbo-sacral	Knees

* Not all areas of body were x-rayed.

here are based on a small series of individuals, and, although they are statistically validated a larger series is necessary to crystallize out the types more clearly and to bring out the smaller differentiae which could not here withstand random sampling validation. Other problems to be investigated include the anthropological status of the mixed arthritis group, the role of race in the frequency of appearance and types of arthritis, the anthropometric correlations with age

of onset of disease, duration of disease, location of the symptoms, and severity of the arthritis. That there is a constitutional factor in arthritis cannot be denied. The anthropological approach is one avenue by which a more complete understanding of the problem of arthritis might be reached.

SUMMARY

This paper reports on the results of an anthropometric comparison between hospital cases of rheumatoid arthritis and degenerative joint disease. Only males are here considered.

The analysis of these data have yielded the following results:

1. The group of rheumatoid arthritics were markedly different in bodily physique from those persons with degenerative joint disease.

2. The physical differences between the two groups were often of such magnitude that the level of statistical significance was reached in the case of a very large number of measures. This occurred in spite of the small size of the series involved, and even after allowances had been made for the age discrepancies between the two groups and the effect of the disease upon various parts of the body.

3. From the profusion of detailed differentiae the degenerative joint disease group may be roughly described as being bigger, heavier, and more lateral in body build than the rheumatoid arthritics. They have a greater overall muscular development and skeletal robusticity than the rheumatoids. No significant differences between the two groups however, could be ascertained in the skeletal structures of the lower extremities.

4. Although it is true that the rheumatoid arthritics are more linear in body build than the degenerative joint disease group, the results of this investigation do not agree with the impressions of many clinicians who stress the linearity and distinctiveness of the rheumatoids. The emphasis should rather be placed on the strong laterality, stocky build, big-boned, big muscled, highly pyknic, and tapering character of the arthritics with degenerative joint disease.

5. The degenerative joint disease group exhibits greater anthropological distinctiveness and homogeneity than the rheumatoids.

6. Degenerative-hypertrophic changes in the hospital cases studied appear to be more common in the physical type represented by the degenerative arthritic series, apart from possible factors of increased trauma due to marked weight and activity.

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ANTHROPOMETRY AND ARTHRITIS

II. Differences between Rheumatoid and Degenerative Joint Disease: Females

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INTRODUCTION

This paper dealing only with the females contains a comparative study of the anthropometry of rheumatoid and degenerative joint disease (osteoarthritis) arthritics. The material, techniques of analysis, and other pertinent information necessary to the fuller understanding of this report are to be found in the parent paper entitled "Anthropometry and Arthritis. I. Differences Between Rheumatoid and Degenerative Joint Disease: Males."

RACE AND NATIONALITY EXTRACTION

When the rheumatoid females are compared with the degenerative joint disease females for nationality extraction (table 1), no significant discrepancies are found between the two groups. As in the males, the strongest elements are the Irish on the one hand, and the English and Scotch on the other. The "Western and Central European mixtures" are the next most important element in the female series, and appear to be somewhat more heavily represented here than in the males.

There is no evidence that the two arthritic female series are significantly divergent in racial composition.

AGE

From the frequency distributions given in table 2 it can be seen that the female degenerative joint disease group is considerably older than the corresponding rheumatoids. The average age for the female degenerative series is 60.0 years against an average of only 38.0 years for the rheumatoids, a difference of 22 years. Thus, the age situation in the female groups is almost exactly the same as for the males. The average age for the rheumatoid males was 37.3 years and for the degenerative joint disease men 56.5 years.

The same age correction method used for the males was adopted here in the case of the females. The averages of the statistically significant measurements and indices have been computed for the twelve oldest rheumatoids and the twelve youngest degenerative joint disease females. The twelve oldest rheumatoid women range in age from 49 to 68 and give an average of 55.0 years, while the twelve youngest degenerative females range from 49 to 65 and have an average

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age of 56.2 years. As in the males, the averages of the measurements for these two groups are to be used to confirm or negate the direction of the differences found in the comparison of the total series.

TABLE 1

Nationality extraction: Females

	Rheumatoid				Degenerative joint disease			
	Paternal		Maternal		Paternal		Maternal	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Old American	3	3.6	2	2.4			2	11.1
Irish	32	38.1	35	41.7	7	38.9	7	38.9
English and Scotch	28	33.3	26	30.9	8	44.4	6	33.3
Scandinavian	3	3.6	4	4.8				
Italian	2	2.4	3	3.6				
Western and Central European Mixtures and others	16	19.0	14	16.7	3	16.7	3	16.7
Totals	84	100.0	84	100.1	18	100.0	18	100.0

TABLE 2

Age frequency distributions: Females

Age	Rheumatoid	Degenerative
	number	*number*
x–19	2	
20–24	6	
25–29	9	
30–34	19	
35–39	12	
40–44	9	
45–49	16	2
50–54	7	4
55–59	2	1
60–64	1	4
65–69	1	6
70–74		1
Totals	84	18
Averages	38.0	60.0

BODY WEIGHT

The body weights at the time of measurement of the two female arthritic series are to be found in table 3. As in the males, we find that the degenerative joint disease group of women is decidedly heavier in body weight than rheumatoid females. The average weight for the degenerative group is 150.3 pounds compared to 125.1 pounds for the rheumatoids. This difference between the

averages of 25.2 pounds is a smaller differential than that of the male groups which amounted to 36 pounds.

No tabulations of the "best weights" of the two female arthritic series are included in this paper owing to the unreliability of such data. In many cases the medical histories failed to contain any note concerning the patients' "best weight" and even when this was recorded the examining physician often referred to the unreliability and vagueness of the figure. In any event, the "best weights" found in the medical histories gave an average of 166.3 pounds for the degenerative joint disease females, against 135.6 for the rheumatoid females, a

TABLE 3

Body weight frequency distributions: Females

WEIGHT	RHEUMATOID (WHEN MEASURED)	DEGENERATIVE JOINT DISEASE (WHEN MEASURED)
lbs.		
70– 79	1	
80– 89	2	
90– 99	5	
100–109	13	
110–119	13	1
120–129	16	2
130–139	15	2
140–149	7	4
150–159	5	3
160–169	3	4
170–179	1	
180–189	1	1
190–199	1	
200–209		1
Totals	83	18
Average	125.1 lbs.	150.3 lbs.

	NUMBER	AVERAGE WEIGHTS (WHEN MEASURED)
Oldest rheumatoids	11	154.2
Youngest degenerative joint disease	10	117.0

difference between the averages of 30.7 pounds. This may suggest that the difference between the "best weights" of the two female arthritic series is slightly larger than the difference between the weights at the time of measurement. However, no significance can be attached to this observation until more reliable information as to the "best weights" of the females is obtained.

GROSS BODY MEASUREMENTS AND PROPORTIONS

Table 4 gives the statistical constants for those anthropometric characters which show significant differences between the two groups of arthritic women. From these data it appears that the rheumatoid females have larger sitting

heights than the group with degenerative joint disease, very much greater spans relative to their statures, and somewhat shorter trunk heights relative to their sitting heights. In absolute dimensions, stature is greater in the rheumatoids

TABLE 4

Gross body measurements and indices: Females

	NUMBER	RANGE	MEAN P.E.	COMPARISON BETWEEN MEANS		
				Diff.	P.E.	Critical ratio
Measures showing significant mean differences:						
Sitting height:						
Rheumatoid	74	76.0– 92.4	84.40 ± .25	1.91	± .62	3.08
Degenerative	17	77.5– 89.4	82.49 ± .57			
Span/stature:						
Rheumatoid	21	94.8–101.3	98.04 ± .25	−3.14	± .52	6.04
Degenerative	14	95.7–106.7	101.18 ± .46			
Trunk height/sitting height:						
Rheumatoid	74	61.5– 69.0	65.73 ± .11	−.68	± .16	4.25
Degenerative	17	64.7– 67.6	66.41 ± .12			
Measures showing no significant differences:						
Stature, span, trunk height, sitting height/stature, trunk height/stature						

TABLE 5

Age comparisons for gross body measurements and indices: Females

	NUMBER	MEAN
Measures showing significant mean differences in original comparisons:		
Sitting height:		
Old rheumatoids	9	83.5
Young degeneratives	12	82.8
Span/stature:		
Old rheumatoids	3	97.9
Young degeneratives	11	101.0
Trunk height/sitting height:		
Old rheumatoids	9	67.0
Young degeneratives	12	66.3

and maximum arm span somewhat shorter. However, the differences between the two groups in stature and span are not statistically significant beyond the error of random sampling. The age comparisons support the directional differences for stature and maximum arm span.

The age comparison data in table 5 confirm the direction of the significant differences in gross body characters in the case of sitting height and the span/stature index, but negates the difference in the trunk height/sitting height index.

TABLE 6

Upper torso measurements and indices: Females

	NUMBER	RANGE	MEAN	P.E.	COMPARISON BETWEEN MEANS		
					Diff.	P.E.	Critical ratio
Measures showing significant mean differences:							
Chest breadth:							
Rheumatoid	76	22.0– 29.9	24.99	± .12	−1.74	± .32	5.44
Degenerative	17	23.0– 30.9	26.73	± .30			
Chest depth:							
Rheumatoid	77	15.3– 25.4	19.05	± .13	−2.10	± .31	6.77
Degenerative	17	18.6– 25.7	21.15	± .28			
Chest circumference:							
Rheumatoid	73	69.0– 96.9	80.55	± .48	−8.31	± 1.15	7.23
Degenerative	17	77.0–103.9	88.86	± 1.05			
Intercostal angle:							
Rheumatoid	65	10.0– 52.0	30.29	± .89	−15.96	± 2.47	6.46
Degenerative	16	23.0– 75.0	46.25	± 2.30			
Chest breadth/stature:							
Rheumatoid	56	13.5– 19.6	15.80	± .10	−1.23	± .20	6.15
Degenerative	17	15.3– 19.6	17.03	± .17			
Chest circumference/stature:							
Rheumatoid	56	43.8– 63.6	50.81	± .37	−5.77	± .72	8.01
Degenerative	17	50.4– 65.3	56.58	± .62			
Sternal/chest breadth:							
Rheumatoid	67	40.6– 75.5	57.38	± .56	4.34	± 1.28	3.39
Degenerative	17	37.1– 65.0	53.04	± 1.15			
Chest index:							
Rheumatoid	76	58.0– 94.9	76.37	± .49	−2.90	± .95	3.05
Degenerative	17	73.0– 88.9	79.27	± .81			
Measures showing no significant differences:							
Biacromial diameter, chest length, sternal length, biacromial/stature, sternal/torsal index, biacromial/trunk height							

UPPER TORSO MEASUREMENTS AND PROPORTIONS

The rheumatoid females are significantly narrower than the degenerative joint disease group in the breadth of the chest, considerably smaller in the depth of the chest, and very markedly smaller in chest circumference. As in the males,

the mean intercostal angle of the rheumatoid females is outstandingly narrower than for the degenerative group. The magnitude of the difference between the rheumatoid and degenerative females in the intercostal angle is illustrated by the fact that while 50 per cent of the degenerative joint disease group have intercostal angles greater than 46 degrees, only 5 out of 84 or 6 per cent of the rheumatoids have correspondingly wide intercostal angles.

With respect to the upper torso proportions it is found that the rheumatoid females compared to the degenerative joint disease group have chest outlines

TABLE 7

Age comparisons for upper torso measurements and indices: Females

	NUMBER	MEAN
Measures showing significant mean differences in original comparisons:		
Chest breadth:		
Old rheumatoids	9	25.5
Young degeneratives	12	26.8
Chest depth:		
Old rheumatoids	9	19.2
Young degeneratives	12	20.9
Chest circumference:		
Old rheumatoids	9	81.5
Young degeneratives	12	89.0
Intercostal angle:		
Old rheumatoids	8	30.6
Young degeneratives	12	44.8
Chest breadth/stature:		
Old rheumatoids	9	16.2
Young degeneratives	12	17.2
Chest circumference/stature:		
Old rheumatoids	9	51.8
Young degeneratives	12	56.9
Sternal/chest breadth:		
Old rheumatoids	9	55.9
Young degeneratives	12	52.7
Chest index:		
Old rheumatoids	9	75.1
Young degeneratives	12	77.8

which are considerably flatter, chest breadths which are narrower relative to the statures, very much smaller chest circumferences in relation to their statures, and distinctly longer sternal length relative to the breadth of their chests. Again as in the males the difference between the two female groups in chest circumference/stature index is especially significant. Thus, while 30 out of 56 or 54 per cent of the rheumatoid females have chest circumferences/stature indices less than 50, none of the degenerative series fall into this category.

The age comparisons in table 7 in every instance confirm the direction of the significant differences just described.

LOWER TORSO MEASUREMENTS AND PROPORTIONS

From the figures in table 8 the rheumatoid females are narrower in hip breadth than the degenerative females, markedly smaller in abdominal circumference, and narrower in hip breadth relative to stature, shoulder breadth, and trunk height. The table of age comparisons support the findings of these significant divergencies in all cases.

UPPER EXTREMITY MEASUREMENTS AND PROPORTIONS

The rheumatoid arthritics are considerably smaller than the degenerative joint disease group in distal humeral breadth (breadth across the epicondyles), much narrower in wrist breadth and in hand breadth. The significantly smaller mean hand index of the rheumatoids indicates a hand shape which is relatively longer and narrower. This is not due to any difference in hand length between the two groups but is the result entirely of the narrower hand breadth of the rheumatoids. The total arm length in relation to the body stature is shorter in the rheumatoids than in the group of degenerative joint disease women.

All the differences here observed are confirmed by the age comparisons in table 11.

LOWER EXTREMITY MEASUREMENTS AND PROPORTIONS

In the lower extremities the rheumatoid females are significantly smaller in the epicondylar breadth of the femur (distal femoral breadth) but not in ankle breadth. As a matter of fact the ankle breadth in the rheumatoids is somewhat larger than in the degenerative group but not significantly so. The calf circumference is smaller in the rheumatoids as is the relation of the calf circumference to the shoulder breadth. The weight discrepancies between the two arthritic groups is certainly in part responsible for these calf circumference differences.

Again as in the males, it is found that the rheumatoid arthritics have very much greater ankle and knee breadths relative to the chest circumferences. This is all the more remarkable in the case of the distal femoral/chest circumference index, inasmuch as the distal femoral breadth was found to be significantly narrower in the rheumatoids than in the degenerative group.

HEAD, FACE AND NECK MEASUREMENTS AND PROPORTIONS

The rheumatoid arthritic females in comparison with the degenerative joint disease females have somewhat smaller neck circumferences, considerably narrower nasal breadths, smaller interocular diameters (distance between the internal palpebral margins), shorter and narrower ears, and longer head and neck heights. The indicial differences indicate the possession by the rheumatoids of narrower heads relative to the cranial heights, and decidedly longer and narrower noses.

The age comparisons in table 15 do not support the significant differences in interocular diameter, ear length, and breadth/height cranial index. The differences, then, between the two arthritic groups for these characters may be ascribed to the discrepancy in their mean ages.

TABLE 8

Lower torso measurements and indices: Females

	NUMBER	RANGE	MEAN	P.E.	COMPARISON BETWEEN MEANS		
					Diff.	P.E.	Critical ratio
Measures showing significant mean differences:							
Bi-iliac diameter:							
Rheumatoid	77	24.3– 35.3	28.77	± .15	−1.54	± .36	4.28
Degenerative	17	26.7– 34.7	30.31	± .33			
Umbilical circumference:							
Rheumatoid	72	54.0– 98.9	68.22	± .64	−14.60	± 1.74	8.39
Degenerative	16	63.0– 97.4	82.82	± 1.62			
Bi-iliac/stature:							
Rheumatoid	57	15.7– 22.8	18.23	± .11	−1.11	± .20	5.55
Degenerative	17	17.6– 20.8	19.34	± .17			
Bi-iliac/biacromial:							
Rheumatoid	76	71.0–101.9	85.19	± .46	−3.14	± .91	3.45
Degenerative	17	81.0– 98.9	88.33	± .79			
Bi-iliac/trunk:							
Rheumatoid	74	44.5– 70.9	51.85	± .32	−3.64	± .73	4.99
Degenerative	17	47.0– 62.4	55.49	± .66			
Measures showing no significant differences:							
Total abdominal length, upper abdominal length, lower abdominal length, upper abdominal/torsal, lower abdominal/torsal, sternal/abdominal, upper abdominal/lower abdominal, bi-iliac/chest breadth							

TABLE 9

Age comparisons for lower torso measurements and indices: Females

	NUMBER	MEAN
Measures showing significant mean differences in original comparisons:		
Bi-iliac diameter:		
Old rheumatoids	9	29.0
Young degeneratives	12	30.2
Umbilical/Circumference:		
Old rheumatoids	9	71.0
Young degeneratives	11	81.9
Bi-iliac/stature:		
Old rheumatoids	9	18.4
Young degeneratives	12	19.3
Bi-iliac/biacromial:		
Old rheumatoids	9	85.4
Young degeneratives	12	87.4
Bi-iliac/trunk:		
Old rheumatoids	9	51.9
Young degeneratives	12	62.2

TABLE 10

Upper extremities measurements and indices: Females

	NUMBER	RANGE	MEAN	P.E.	COMPARISON BETWEEN MEANS Diff.	P.E.	Critical ratio
Measures showing significant mean differences:							
Distal humeral breadth:							
Rheumatoid	74	54.0–71.0	60.68	± .27	−3.21	± .87	3.69
Degenerativo	18	58.0–79.0	63.89	± .83			
Wrist breadth:							
Rheumatoid	54	42.0–58.0	49.91	± .31	−2.03	± .49	4.14
Degenerative	18	49.0–60.0	51.94	± .38			
Hand breadth:							
Rheumatoid	45	64.0–79.0	70.67	± .40	−3.39	± .62	5.47
Degenerative	16	70.0–81.0	74.06	± .48			
Arm/stature:							
Rheumatoid	21	36.5–40.0	38.43	± .12	−1.06	± .23	4.61
Degenerative	14	37.5–42.0	39.49	± .20			
Hand index:							
Rheumatoid	44	36.9–47.3	41.64	± .22	−1.87	± .38	4.92
Degenerative	16	39.9–47.0	43.51	± .31			
Measures showing no significant differences:							
Hand length, arm length, forearm length, upper arm length, forearm/upper arm							

TABLE 11

Age comparisons for upper extremities measurements and indices: Females

	NUMBER	MEAN
Measures showing significant mean differences in original comparisons:		
Distal humeral breadth:		
Old rheumatoids	9	61.4
Young degeneratives	12	62.7
Wrist breadth:		
Old rheumatoids	6	51.5
Young degeneratives	12	51.9
Hand breadth:		
Old rheumatoids	6	73.5
Young degeneratives	11	74.2
Arm/stature:		
Old rheumatoids	3	38.0
Young degeneratives	11	39.4
Hand index:		
Old rheumatoids	6	42.8
Young degeneratives	11	43.4

TABLE 12

Lower extremities measurements and indices: Female

	NUMBER	RANGE	MEAN	P.E.	COMPARISON BETWEEN MEANS		
					Diff.	P.E.	Critical ratio
Measures showing significant mean differences:							
Distal femoral breadth:							
Rheumatoid	41	79.0– 97.0	86.88	± .41	−5.30	± 1.15	4.61
Degenerative	11	81.0–102.0	92.18	± 1.07			
Calf circumference:							
Rheumatoid	70	23.0– 37.9	29.79	± .20	−3.94	± .42	9.38
Degenerative	16	30.5– 37.9	33.73	± .37			
Calf circumference/biacromial diameter index:							
Rheumatoid	61	72 –107	89.06	± .63	−8.88	± 1.26	7.05
Degenerative	16	86 –108	97.94	± 1.09			
Ankle breadth/chest circumference index:							
Rheumatoid	53	62 – 89	77.02	± .55	7.67	± 1.09	7.04
Degenerative	17	60 – 80	69.35	± .94			
Distal femoral/chest circumference index:							
Rheumatoid	39	92 –127	108.66	± .71	4.93	± 1.42	3.47
Degenerative	11	93 –114	103.73	± 1.23			
Measures showing no significant differences:							
Ankle breadth, tibiale-spherion length, lower extremities length, intermembral index, tibiale/stature, calf circumference/chest circumference index							

TABLE 13

Age comparisons for lower extremities measurements and indices: Females

	NUMBER	MEAN
Measures showing significant mean differences in original comparisons:		
Distal femoral breadth:		
Old rheumatoids	5	88.0
Young degeneratives	10	92.2
Calf circumference:		
Old rheumatoids	8	30.8
Young degeneratives	11	33.8
Calf circumference/biacromial diameter index:		
Old rheumatoids	9	90.5
Young degeneratives	12	97.7
Ankle breadth/chest circumference index:		
Old rheumatoids	9	75.0
Young degeneratives	12	69.0
Distal femoral/chest circumference index:		
Old rheumatoids	9	108.0
Young degeneratives	12	103.6

TABLE 14

Head, face and neck measurements and indices Females

	NUMBER	RANGE	MEAN	P E	COMPARISON BETWEEN MEANS Diff	P E	Critical ratio
Measures showing significant mean differences							
Neck circumference							
Rheumatoid	75	28 2-35 6	31 51	± 13	−1 64	± 38	4 32
Degenerative	17	29 4-37 1	33 15	± 36			
Nose breadth							
Rheumatoid	84	25 0-39 0	31 54	± 20	−3 18	± 37	8 59
Degenerative	18	30 0-38 0	34 72	± 31			
Interocular							
Rheumatoid	84	26 0-39 0	32 01	± 20	−1 66	± 49	3 39
Degenerative	18	28 0-39 0	33 67	± 45			
Ear length							
Rheumatoid	84	48 0-70 0	59 56	± 31	−5 05	± 93	5 43
Degenerative	18	57 0-81 0	64 61	± 88			
Ear breadth							
Rheumatoid	84	28 0-40 0	33 92	± 19	−2 69	± 33	8 15
Degenerative	18	34 0-40 0	36 61	± 27			
Head and neck height							
Rheumatoid	62	24 0-31 7	28 43	± 12	1 28	± 31	4 13
Degenerative	17	22 8-29 9	27 15	± 29			
Head and shoulder height							
Rheumatoid	63	20 7-30 5	26 36	± 18	1 32	± 42	3 14
Degenerative	17	20 7-29 9	25 04	± 38			
Breadth/height cranial index							
Rheumatoid	56	68 0-94 9	80 47	± 44	2 67	± 86	3 10
Degenerative	17	71 0-86 9	77 80	± 74			
Nasal index.							
Rheumatoid	84	44 0-73 9	58 43	± 44	−5 24	± 1 33	3 94
Degenerative	18	48 0-80 9	63 67	± 1 25			
Measures showing no significant differences							
Head circumference, head length, head breadth, minimum frontal, bizygomatic diameter, bigonial, total face height, upper face height, nose height, biocular diameter, gonial angle, head height, length/breadth cranial, length/height cranial, fronto parietal index, cephalo/facial index, zygo/frontal index, fronto/gonial index, ocular/zygomatic index, total facial index, upper facial index, ear index, zygo/gonial index							

SOMATOTYPES

Unfortunately, accurate techniques for the somatotyping of women have not yet been fully developed (1). For this reason no tabulation of the somatotype ratings of the females in the two arthritic groups has been included in this paper. Nevertheless, the ratings made by this observer on the females have provided certain general impressions of their body build types.

TABLE 15

Age comparisons for head, face, neck measurements and indices: Females

	NUMBER	MEAN
Measures showing significant mean differences in original comparisons:		
Neck circumference:		
Old rheumatoids	9	31.6
Young degeneratives	12	33.1
Nose breadth:		
Old rheumatoids	12	33.0
Young degeneratives	12	34.1
Interocular:		
Old rheumatoids	12	33.8
Young degeneratives	12	32.8
Ear length:		
Old rheumatoids	12	62.2
Young degeneratives	12	62.6
Ear breadth:		
Old rheumatoids	12	35.7
Young degeneratives	12	36.5
Head and neck height:		
Old rheumatoids	9	27.5
Young degeneratives	12	27.1
Head and shoulder height:		
Old rheumatoids	9	25.7
Young degeneratives	12	25.0
Breadth/height cranial index:		
Old rheumatoids	9	77.0
Young degeneratives	12	78.4
Nasal index:		
Old rheumatoids	12	59.8
Young degeneratives	12	63.1

In the rheumatoids the dominant type is one which is low in the pyknic component, low to medium in the somatic bone-muscle component, and high in the leptic or linear element. Of lesser importance are two body build types of approximately equal frequency. The first is high in the pyknic component, high to medium in the somatic, and low in the leptic element. The second is medium in the pyknic, medium in the somatic, and medium to high in the linear component. There are other types as well but they occur rather infrequently.

In the degenerative joint disease group virtually all the women fall into a

single general body build type. This type is most often medium in the pyknic component but may occasionally be quite high and less often fairly low. The somatic component is very strongly represented, while the linear element is definitely on the low side. There is a small residual group in the degenerative series which is low to medium in the pyknic, medium in the somatic, and high in the linear elements.

On the whole it appears that there is a good deal of similarity in body build types between the males and females of both the rheumatoids and the groups with degenerative joint disease.

We have already seen that individuals with degenerative joint diseases are characterized by very strong somatic or bone-muscle development. Accordingly, since women are on the whole less well-developed in the somatic or bone-muscle element than men, it is interesting to consider whether this may have any connection with the fact that proportionately fewer women than men develop degenerative joint disease.

COMPARISON BETWEEN MALES AND FEMALES

The extent of similarity of the males and females in their anthropometric differentiation between rheumatoid arthritics and individuals with degenerative joint disease, is clearly indicated by the data in table 16. In this table are listed 51 anthropometric characters which showed statistically significant differences in the comparison of the two arthritic groups. Out of this number 25 or about 50 per cent of these anthropometric characters showed significant differences in the same direction in both the males and females. There were no cases of significant differences in the opposite direction. Fourteen or about 28 per cent of anthropometric characters showed significant differences in the case of the males only, but the direction of the differentiation in the females in all but two of these instances was always the same in the males. In 12 or about 24 per cent of anthropometric characters which showed significant differences between the two arthritic groups in the females only and not in the males, the direction of the differences in the males was the same in all cases.

There can be no question that the anthropometric differentiation between the two arthritic groups is strikingly alike in both males and females. This similarity between the two sexes is all the more remarkable in view of the small size of some of the groups involved in the comparisons.

COMPARATIVE DATA

The only study of rheumatoid and degenerative joint disease arthritics in which anthropometric data are available is that of Kovacs and Hartung (2). This work deals with a morphological comparison between 50 rheumatoid and 50 osteoarthritic females (degenerative joint disease). Apart from the means, no statistical constants are given which would enable the reader to estimate the sampling error and test the validity or significance of the differences between the anthropometric characters.

In spite of this deficiency a comparison of the means between the rheumatoid

TABLE 16

Comparison of males and females for significant anthropometric differences between rheumatoid arthritis and individuals with degenerative joint disease

SIGNIFICANT DIFFERENCES IN BOTH ♂ AND ♀ AND IN SAME-DIRECTION	SIGNIFICANT DIFFERENCES IN BOTH ♂ AND ♀ AND IN OPPOSITE DIRECTION	SIGNIFICANT DIFFERENCES IN MALES ONLY	DIRECTION OF FEMALE	SIGNIFIANT DIFFERENCES IN FEMALES ONLY	DIRECTION OF MALE
Age		Span	Same	Sitting height	Same
Body weight		Interpapillary diam.		Trunk hght./sit. hght. ind.	Same
Span/stature index		Bi-iliac/chest br.	Same	Chest index	Same
Chest breadth		Hand length	Same	Arm/stature index	Same
Chest depth		Forearm length	Opp.	Bi-iliac/biacromial ind.	Same
Chest circ.		Forearm/upper arm index	Opp.	Distal femoral br.	Same
Intercostal angle		Biacromial/trunk hght. ind.	Same	Interocular	Same
Chest br./stature index		Head circ.	Same	Ear breadth	Same
Chest circ./stature index		Bizygomatic diameter	Same	Head and neck hght. ind.	Same
Sternal/chest br. index		Bigonial	Same	Head and shoulder hght. ind.	Same
Bi-iliac diameter		Fronto/gonial index	Same	Breadth/height cranial ind.	Same
Umbilical diameter		Zygo/gonial index	Same	Nasal index	Same
Bi-iliac/stature index		Ocular/zygomatic index	Same		
Bi-iliac/trunk index		Ear index	Same		
Distal humeral br.					
Wrist breadth					
Hand breadth					
Hand index					
Calf circumference					
Calf circ./biacromial diameter index					
Distal femoral/chest circ. index					
Ankle br./chest circ. index					
Nose breadth					
Ear length					
Neck circ.					

and degenerative groups by simple inspection reveals a marked similarity with the results obtained in this paper. In both Kovacs and Hartung's work and in this study the rheumatoid arthritics are found to be considerably younger in mean age, much lighter in body weight, shorter in arm span, shorter in head length, smaller in head circumference, longer in neck length, and smaller in neck and abdominal circumferences. The rheumatoids are considerably smaller in both the antero-posterior and lateral dimensions of the chest, as well as in chest circumference. Similarity between both investigations are to be noted in the narrower hips of the rheumatoids and in a number of indices computed from the above measurements. The only disparities observed between Kovacs and Hartung's study and the material reported in this paper occur in the case of head breadth, cranial index, and the chest index. Kovacs and Hartung find the rheumatoids with broader heads, rounder heads and rounder chests than in the degenerative joint disease group.

SUMMARY

This paper contains the analysis of an anthropometric comparison between rheumatoid arthritics and group with degenerative joint disease. This report deals only with females.

The results plainly indicate that the female rheumatoid arthritics are markedly different in bodily physique from those women with degenerative joint disease.

The form and extent of this differentiation between the two female arthritic groups closely parallels that found for the males.

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THE PHYSIOLOGICAL EFFECTS OF CARBON DIOXIDE ON THE ACTIVITY OF THE CENTRAL NERVOUS SYSTEM IN MAN

With Special Reference to the Problem of High Altitude Flying. A Review

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All research in physiology has had to recognize that the human body functions as a whole and that no one part of it can be considered as a separate entity, nor can the influence of any agent be studied by its effects on a single part of it in isolation from the rest. The interplay of all the systems of the body is finely adjusted to produce a balance between them and the unbalance of any one organ of the body will have far reaching effects throughout the whole system.

In the present review the central problem is that of the influence of carbon dioxide on the central nervous system in man, a field extensive enough in itself, but reaching out into problems concerning the heart, the lungs, the blood vessels and the muscles.

For the purposes of this review, and in an attempt to set up some limits to the field under study, the physiological effects of carbon dioxide on the central nervous system have been roughly classified. They are both direct and indirect in their mechanism of stimulation.

(1) Direct stimulation of the respiratory centers in the medulla and spinal cord.

(2) Stimulation of the special nerve endings (chemo-receptors) in the carotid bodies and aortic arch, with the resultant vasodilator action on the cerebral blood vessels.

(3) Direct stimulation of the vasomotor centers in the hypothalamus, midbrain and medulla.

(4) Direct action on the cerebral blood vessels.

(5) Effect on the affinity of blood for oxygen.

THE ACTION OF CARBON DIOXIDE ON THE RESPIRATORY CENTERS IN THE MEDULLA AND SPINAL CORD

The respiratory center is very sensitive to changes in CO_2 tension in alveolar air. During the breathing of normal air the alveolar CO_2 pressure is usually approximately 40 mm. Hg; a rise of 2.5 mm. Hg in this alveolar CO_2 pressure (produced by inspiring air with 4 per cent CO_2 added) will approximately double the alveolar ventilation (Campbell, Douglas and Hobson (1)).

The question as to whether the HCO_3 ion has a specific action on the respiratory centers, i.e., not only by the increase in H ion concentration, was for a long time a controversial one. Haldane (2) held that the pH of arterial blood was the controlling factor, and not CO_2 specifically. This view was supported by the work of Winterstein (3) and of Hasselbach (4, 5).

That CO_2 has a specific stimulating effect on the respiratory centers was suggested by the work of Lacquer and Verzar (6), and of Hooker, Wilson and Connett (7) with animals in which they showed that perfusing fluids of high CO_2 tension had a far greater stimulating effect than fluids of the same pH but with low CO_2 tensions. This theory was supported by Scott (8, 9) who showed that when the H ion concentration of the blood is first of all lowered by injecting alkali intravenously, hypernea still develops in proportion as the CO_2 accumulates in the inspired air, even though the H ion concentration, when hypernea is at its highest, is below that of normal blood. The work of Collip (10), of Dale and Evans (11), and of Myerson, Loman, Edwards and Dill (12) provided further support.

The specificity of CO_2, however, is due to the fact that cell membranes are more permeable to CO_2 than to other acids (Jacobs (13)) so that the pH of the interior of the cell is lowered. The work of Gesell (14) has cleared up this point and shown conclusively that it is the pH of the cells of the respiratory centers which is the stimulus to respiration.

Thus the respiratory centers are controlled by the pH of their cells with the resultant oxidative changes, not by the pH of the arterial blood, and the specific action of CO_2 is by virtue of its rapid penetration of cell membranes.

THE INFLUENCE OF ANOXIA ON THE RESPIRATORY CENTERS

In anoxia the oxygen-lack acts as a relatively weak stimulus to the respiratory centers and pulmonary ventilation is increased. For oxygen-lack to act as a respiratory stimulus the O_2 content of the inspired air must fall to 13 per cent (Haldane and Priestley (17)) although some effects were obtained by Ellis (18) at 18 per cent. At a level of 13 per cent O_2 in the inspired air the minute respiratory volume increases by 5 or 6 liters (Schneider (19)); this washes out the CO_2 from the lungs and blood, so that the CO_2 tension in the arterial blood falls. At high altitudes the continuous anoxia causes a severe loss of the CO_2 necessary for stimulation of the respiratory centers (Armstrong (20)) and Van Liere (21)).

At low altitudes when air is breathed the alveolar tension of CO_2 and the per minute volume of breathing vary proportionally, so that the CO_2 tension remains practically constant. The opposite is true at high altitudes where the alveolar CO_2 tension decreases with increasing altitude (21).

To summarize: any impairment of the oxygen supply to the cells of the brain and medulla will interfere with their oxidative processes and will delay the formation and removal of the intermediary products of this catabolism. Since the respiratory centers are stimulated by the change in hydrogen ion concentration of their own cells (and not by that of their blood supply) this accumulation of acid metabolites will lower the pH of the centers with a resultant increase in respiration.

Administration of oxygen will complete the catabolism of the intermediary products in the cells (chiefly lactic acid) to the end product, CO_2, which diffuses more rapidly than lactic acid through the cell membranes and is carried away by the blood.

INFLUENCE OF CARBON DIOXIDE AND OF ANOXIA ON THE CHEMORECEPTORS IN THE CAROTID BODIES AND AORTIC ARCH

Rise or fall in the CO_2 pressure of the blood circulating through the carotid body has the same effects upon respiration as it does on the centers in the medulla and spinal cord (15) and the factor in the control of the chemoreceptors in the carotid body is again the intracellular pH (16).

Experiments on the intact animal indicate that anoxia stimulates the respiratory centers by acting on the sensory nerve endings in the aortic arch and carotid bodies (Cordier and Heymans (22), Heymans and Bouckaert (23), Comroe and Schmidt (24), and Bernthal (25)). These centers are more sensitive to anoxia and less sensitive to CO_2 than are the respiratory centers (Wright (26) and Schmidt (27)). Selladurai and Wright (28) believe this to be the only mode of action of oxygen-lack on the respiratory center, and that there is no direct stimulation if the sinus nerves and vagi are cut.

Asmussen and Chiodi (29) hold that it is local oxygen-want in the cells of the carotid body which is the stimulus to overventilation in anoxia. It is the O_2 pressure and not the O_2 content of the blood which is the deciding factor, e.g. with low alveolar O_2 and low O_2 content of the arterial blood (38 mm. Hg) as produced by inhaling 10 per cent O_2, there will be overventilation even if there is no exertion. If, however, the alveolar O_2 is kept normal, but the O_2 combining power of the Hb is reduced, by inhaling carbon monoxide to the same arterial O_2 content as before (38 mm. Hg), there is no overventilation.

Gesell (15) holds that the stimulus to overventilation in anoxia is the result of the increased intracellular acidity and impaired oxidation in the cells of the aortic and carotid bodies.

Comroe and Schmidt (24) agree that these centers respond to interference with oxidations within them, but believe that the whole system of carotid body reflexes constitutes an accessory mechanism, rather than an essential part of the normal respiratory regulating system, and that they only come into play in conditions of anoxemia and CO_2 excess, respiratory centers in the medulla being more powerful than the carotid reflexes in controlling pulmonary ventilation.

THE EFFECT OF CARBON DIOXIDE ON THE VASOMOTOR CENTERS

The brain and brain stem have a finely adjusted system for maintaining a constant blood supply irrespective of fluctuations in the systemic arterial pressure (Schmidt (30), and Forbes and Cobb (31)), i.e., the brain regulates its own circulation locally through its vasomotor centers (Lennox and Gibbs (32)).

The most important control of cerebral blood flow is chemical. The cerebral vascular bed is extremely sensitive to changes in arterial CO_2 tension, and also to a lesser extent to decrease in O_2 tension (Mathison (33), Gellhorn and Lambert (34), Gibbs, Gibbs and Lennox (35), Wolff (36), and Bronk and Gesell (37)).

The effect of this extreme sensitiveness of the vasomotor centers to slight changes in CO_2 is to produce a very steady condition of the blood supply to the brain (37).

This fine balance of the blood supply found in the cortex exists also in the brain stem (Penfield and Erickson (38)). Schmidt and Pierson (39) using a thermocouple to measure changes in blood flow in the medulla of cats found a definite and powerful regulation of medullary blood vessels by CO_2 excess. The influence of CO_2 appeared to be practically specific, for pH changes per se had little effect. CO_2 is essential to the normal functioning of the vasomotor centers, as has been shown by Henderson (40) and by Dale and Evans (11).

THE EFFECT OF ANOXIA ON THE VASOMOTOR CENTERS

The effects on cerebral blood flow of changes in O_2 tension are much less marked than those due to changes in CO_2 tension (Gibbs, Gibbs and Lennox (35) and Schmidt and Hendrix (41)).

Moderate changes of the O_2 tension of the cerebral blood do not cause significant alterations in the cerebral circulation (42), but in acute anoxia the oxygen deficiency produces vasodilation of the medullary and hypothalamic vessels with a resultant increase in volume of the blood flow to the medulla oblongata and hypothalamus (Schmidt (43) and Schmidt and Pierson (39)). The parietal cortex is especially sensitive to O_2 lack (44). Severe anoxia also produces a dilation in the pial blood vessels (45). It has been shown in man, by Lennox and Gibbs (46), that although alterations in the O_2 tension of arterial blood have less effect than CO_2 changes, in severe anoxaemia there is an increased blood flow both to the brain and to the extremities.

Increased circulation by itself is unable to cope with anoxaemia, but the concurrent increase in ventilation helps to avoid an excessive reduction in alveolar O_2 (15).

The stimulant effects of anoxia on respiration and circulation are due much more to reflexes from the aortic and carotid bodies than to direct stimulation of the respiratory centers in the medulla and spinal cord (47) whereas CO_2 effects are more central (26, 27).

DIRECT ACTION OF CARBON DIOXIDE ON THE CEREBRAL BLOOD VESSELS

Rise in CO_2 tension in the cerebral tissue causes an immediate dilation of the local blood vessels and an increase in the rate of blood flow through the brain; this has been demonstrated by direct observation of cerebral arteries through an artificial window in the cat's skull (Wolff and Lennox (45)) and also by arterial-venous blood gas differences (46, 48) and by the use of a thermocouple blood flow recorder in the internal jugular vein of man during the inspiration of 10 per cent CO_2 and 90 per cent O_2 (35). Decrease in CO_2 tension caused by hyperventilation has the opposite effect.

Lennox and Gibbs (46) contrasted the arterial-venous differences in man, using samples from the brachial artery and internal jugular vein. The arterial-venous difference in O_2 content was less when breathing air rich in CO_2, and they concluded from this that the cerebral blood vessels were dilated.

Similar results were obtained in dogs by Irving and Welch (48) who found the blood flow through the brain to increase by two to four times when 10 per cent CO_2 was added to the inhaled oxygen. They calculated the blood flow from the

arterial-venous differences. The arterial-venous difference in O_2 content of the blood is less when CO_2 is breathed because there is only a small oxygen loss from the blood during its more rapid passage through the brain. In the blood leaving the brain there is a low but comparatively constant O_2 tension as compared with the O_2 tension of venous blood leaving the extremities (Lennox (49)). That this small oxygen loss from the blood in its passage through the brain is due to the more rapid cerebral blood flow and not to decreased O_2 utilization is shown by the experiments of Lennox, Gibbs and Gibbs with the blood flow recorder.

The inhalation of increased amounts of CO_2 (10 per cent) has also been shown to dilate the arterioles of the human retina (Cobb and Fremont-Smith (50)), as shown by the color of the retinal veins which become bright red.

There is some evidence which indicates that CO_2 acts on the muscle tissue of the blood vessel walls directly (36) and not upon the nerve endings in the vessels (Cow (51)).

During normal functional activity of any cortical area, the blood flow through that part of the cortex is more rapid. This has been demonstrated by the work of Cobb and Talbott (52) in the capillaries of the olfactory bulb in cats, by Fietelberg and Lampl (53) in the motor regions of cats' brains, and by Gerard and Scrota (54) in the optic pathways of animals on illuminating the eyes, and is supported by an observation of Fulton's (55) on man; Fulton observed an increased bruit over the site of an occipital haemangioma in a man when he attempted to read small print.

These increases in blood flow would seem to be due to vasodilation resulting from the presence in the tissue of CO_2 produced by the increased activity of ganglion cells (38), i.e., they provide for increased blood flow at the time of functional increase and guard against the local anoxia which might otherwise occur.

THE EFFECT OF CO_2 ON THE AFFINITY OF BLOOD FOR OXYGEN

The dissociation curve of oxyhaemoglobin is greatly influenced by the CO_2 tension and this factor comes in when the increased rate of respiration which follows anoxia washes out the CO_2 from the lungs. At low CO_2 tensions the oxyhaemoglobin of the blood clings more closely to its oxygen, so that the tissues are starved by reason of their inability to dissociate enough oxygen from the arterial blood (21).

This phenomenon is known as the Bohr effect, i.e. when the CO_2 of the blood is below normal as a result of increased lung ventilation, the blood has a greater affinity for oxygen and consequently becomes more highly saturated as it passes through the lungs. At the same time, however, the blood holds more tightly to its oxygen so that it is not given off so readily to the tissues and the net result is a greater tissue anoxia (20). This is generally held to be an effect due to the H ion rather than to CO_2 specifically.

LOSS OF CONSCIOUSNESS DUE TO ANOXIA

In the following discussion the word "consciousness", a word which is in general very loosely used, will be taken in the sense used by Cobb (56), i.e., awareness of self and of environment, and the ability to discriminate.

Types of anoxia which can cause loss of consciousness are syncope and low O_2 tension in the blood, and there is possibly a third type, i.e., spasm of the cerebral blood vessels.

In a series of 22 human subjects, Lennox, Gibbs and Gibbs (57) showed that a rapid lowering of the oxygen supply to the brain always caused loss of consciousness when the O_2 tension fell below 24 per cent in the internal jugular vein. There was no loss of consciousness provided the O_2 tension did not fall below 30 per cent. A degree of anoxemia sufficient to produce unconsciousness is attended by increased cerebral circulation (42).

Of all the tissues in the body, nervous tissue is the least capable of withstanding oxygen-want. In man, 20 seconds of severe anoxemia produce harmful effects on the brain as evidenced by convulsive seizures (Cobb and Forbes (58)) and (Lennox and Cobb (59)).

Hypernea severe enough to cause Cheyne-Stokes breathing and loss of consciousness in normal subjects has been demonstrated by Schmidt and Hendrix (41). Their subjects voluntarily overbreathed until so much CO_2 was washed out that they lost consciousness. If 5 per cent CO_2 were present in the inspired air, it was impossible for them to overbreathe themselves into unconsciousness. Schmidt and Hendrix cite these experiments as an example of loss of consciousness due to spasm of the cerebral vessels. It must be remembered, however, that severe hyperventilation not only reduces the CO_2 but the oxygen saturation of the blood leaving the brain, which may be reduced by half (60).

One physiological stage between complete consciousness and unconsciousness is sleep. In sleep there is no cerebral anemia: Gibbs, Gibbs and Lennox (61) have shown that there is no significant change in cerebral blood flow during sleep in man, but there is a slight increase in CO_2 in the blood, and respiration becomes periodic.

The various stages of sleep can be followed by a study of the brain potentials. Davis, Loomis and co-workers (62–72) and Blake (73, 74, 75) have shown that with light sleep the alpha rhythm is replaced by slow 6 cycle waves mixed with spindles of 14 cycle. With deeper sleep the rhythm becomes slower and slower until finally it is almost a baseline and shows the same absence of electrical activity that is seen in coma.

Other studies of brain potentials during sleep and states of unconsciousness have been made by Knott (76) and by Travis (77).

Anoxia affects the various parts of the brain in relation to their vulnerability to O_2 changes. The cortex is first to be affected, then the upper portion of the brain stem, and finally the medulla. (Heymans, (78)). This order of progression has been shown to be the same as that for oxygen utilization (Dixon and Meyer (79)) and is followed also by changes in electrical activity (Sugar and Gerard (80)).

THE EFFECT OF CO_2 ON BRAIN POTENTIALS

The electrical potentials of the brain give perhaps the most informative data about the reactions of brain cells to various changes in their environment.

A decrease in the CO_2 tension of the arterial blood causes a decrease in the rate of the brain waves (60). In man, voluntary hyperventilation, which washes out CO_2, results in high voltage slow waves. This response to overventilation has been confirmed by use of the Grass analyser (81), and is held by Gibbs, Gibbs, Lennox and Nims (82) to be due to the decrease in CO_2 in the cerebral blood (as determined in blood samples from the internal jugular vein), and not to anoxia secondary to vasoconstriction.

Davis and Wallace (83) examined the effects of standardized hyperventilation on the brain potentials in man, and from the analysis of blood CO_2 and pH in blood samples from the finger, concluded that the presence of the slow brain potentials was not dependent upon the absolute value of the blood pH. They suggested that the slow brain potentials produced by hyperventilation were probably the result of vasoconstriction. Hyperventilation with oxygen produced less alteration in the brain potentials than did hyperventilation with air maintained at 20 per cent O_2 content.

In experiments on 2 patients who gave large quantities of delta waves on hyperventilation, Schwab (84) was able to obliterate these waves by having the subjects inhale amyl nitrite in small quantities; apparently the vasodilation so caused counteracted the poor blood supply to the brain, but this experiment in itself is not critical in deciding whether anoxia or washing out of CO_2 is the dominant factor.

Davis now believes (personal communication) that the more recent evidence of Gibbs, Gibbs, Lennox and Nims (82) demonstrates the inadequacy of his anoxia hypothesis as an explanation of the production of slow potentials by hyperventilation and points clearly to the primary importance of washing-out of CO_2.

Contrarily, increase in the CO_2 tension of arterial blood increases the average frequency of the brain potentials (60). This effect can be demonstrated in the degree of CO_2 tension increase attained by holding the breath (Gerard (85)). Inhalation of a mixture of 10 per cent CO_2 and 90 per cent O_2 decreases the voltage of the slow waves produced by hyperventilation (and also of the slow waves seen in petit mal). This can be shown to be an effect of the CO_2 because O_2 alone does not abolish these waves (Lennox, Gibbs and Gibbs (86)).

EFFECT OF ANOXIA ON BRAIN POTENTIALS IN MAN

Berger (87) in his experiments on man found that anoxia induced by rebreathing respired air from which the CO_2 has been removed made the brain potentials more irregular and that after about seven minutes, larger waves appeared. This was confirmed by Gibbs and Davis (88) on human subjects breathing pure nitrogen, but the anoxemia had to be severe before any significant slowing took place (60, 81, 80). A study of these changes was made by Davis, Davis and Thompson (90); their subjects breathed gas mixtures containing 7.8 to 11.4 per cent O_2; there was a slight initial increase in the average voltage of the brain waves, but later it decreased and shorter trains of alpha waves occurred. By the time cyanosis developed there were large irregular delta waves which

dominated the record when consciousness was lost. These delta waves disappeared with the first breath of room air, and the record was back to normal in two minutes.

In milder degrees of anoxemia before the appearance of the delta waves, there is an increase in the frequency of the alpha waves (Hoagland (91)); a direct relationship between the O_2 utilization of the brain and the frequency of the alpha rhythm has been demonstrated by Himwich (92) and an inverse relationship between O_2 utilization and delta frequency. Lindsley and Rubenstein (93) and Ross and Schwab (94) found a direct relationship in man between the frequency of alpha waves and the total calories per hour.

From his experiment with pyrexia Hoagland (95) concludes that the alpha rhythm is directly determined by the local respiration of the cells of the cortex. He believes that the local respiration continually builds up electrical potentials which discharge as oscillations at critical potential values. The alpha frequency would thus be proportional to the respiration rate and therefore slower in anoxia.

Since dextrose is the principal substrate in cerebral metabolism, hypoglycemia is an adequate method for reducing the O_2 utilization in the brain. Himwich and his coworkers (96) showed that on insulin injection the march of clinical symptoms was accompanied by an increasing cerebral A-V O_2 difference and a decrease in the alpha frequency. A similar correlation between brain metabolism and brain potentials was found in cretins in whom the A-V O_2 differences were reduced by thyroid administration; as a result there was an average increase of 32 per cent in cerebral metabolism and a concomitant increase in the energy level of brain waves in the 7 to 11 cycle band (Himwich, Daly, Fazekas and Herrlich (97)).

In simulating the anoxia of high altitudes by use of a pressure chamber, Lyman, Carlson and Benson (98) have differentiated between the effects on brain potentials of low barometric pressures with and without the oxygen mask. Two subjects taken in a pressure chamber to the equivalent of 35,000 feet, and one taken to 40,000 feet showed no change in their brain waves when breathing oxygen with the oxygen mask. Without the mask, however, changes were seen in the brain wave at the equivalent of 18,000 feet; the waves became irregular, there was a decrease in amount of alpha activity and voltage, and a general flattening of the record.

Attempts have been made to study the effects of oxygen-lack in man by breathing an anesthetic mixture of O_2 and nitrous oxide but Derbyshire has been able to show that the anoxic results are less when breathing 5 per cent O_2 with 95 per cent N_2O than when 5 per cent O_2 with 95 per cent nitrogen is used (Personal communication). Although the mechanism underlying this result is as yet unexplained, it is an indication that this method of inducing anoxia may not be strictly comparable to pressure chamber methods.

It is certainly clear that the 'inert' component of any gas mixture used in the study of the effects of oxygen-lack must be taken into account. Several workers (99, 100, 101) have shown the beneficial sedative action of using helium instead

of nitrogen as the inert component of O_2 mixtures in deep sea diving, and Coh and Katzenelbogen (102) have concluded that helium may assist in the main tenance of an optimum O_2 supply to the cortex.

THE EFFECT OF CO_2 AND O_2 CHANGES ON THE CORTICAL POTENTIALS IN ANIMAL

There has been a great deal of work in this field, and it confirms in all esser tials the results obtained in man. The chief contributions will be found in th publications of the following workers: Bailey and Bremer; Bartley and Bishop Beecher, McDonough and Forbes; Bremer; Bremer and Thomas; Derbyshir Rempel, Forbes and Lambert; Dusser de Barenne, Marshall, McCulloch an Nims; Gerard and Libet; Hoagland, Himwich, Campbell, Fazekas and Had dian; Libet, Fazekas and Himwich; Simpson and Derbyshire; and Sugar an Gerard (103–115).

THE ADMINISTRATION OF CARBON DIOXIDE IN ANOXIA

The most outstanding advocate for the use of CO_2 to counteract the effects anoxia has been Henderson (116). He has pointed out that there are con ditions due to prolonged deficiency of O_2 that are effectively counteracted by th administration of CO_2 and he has urged the recognition of CO_2 as a 'tonic' and stimulant.

One of the first observers of this effect was Mosso (117) who found that h could increase the resistance of human subjects to low barometric pressure b allowing them to inhale 2 to 5 per cent CO_2. This was confirmed in animals b Margaria (118) and in men by Talenti (119) who was able to extend the 'ceilin of resistance' to 39,000 feet by the addition of 3 per cent CO_2 to the inhaled O the same subjects had had their first symptoms at 18,000 feet when breathin air. A further improvement was demonstrated with 7 to 10 per cent CO_2 adde to the O_2. Margaria (118) thought that CO_2 alleviated the effects of the lo barometric pressure, but that it would not have the same effect on the oxyger want produced by the addition of N_2 at normal atmospheric pressure. Subse quent work has proved him mistaken in this belief (120).

Aggazzotti (121) found that 67 per cent O_2 with 13 per cent CO_2 in the inspire gas mixture enabled men to reach simulated heights of 43,500 feet in the pressur chamber without experiencing any distress. This confirmed the results of h previous experiments on monkeys (122). The more recent work in the pressur chamber experiments at Saint-Cyr with inhaled mixtures containing a ratio c 13 CO_2:100 O_2 gave marked beneficial results at 24,000 feet (123).

Studies on men living at high altitudes have led several observers to the sam conclusion. Hasselbach and Lindhard (124, 125) noted that symptoms of moun tain sickness were reduced by inhalations of proper dilutions of CO_2, and th same observation led Winterstein (126) to the conclusion that CO_2 is helpful i maintaining respiration and promoting O_2 absorption at great altitudes.

On Pike's Peak Childs, Hamlin and Henderson (127) found that the inhale tion of small amounts of CO_2 kept their respiration more regular, modified th rise in systolic blood pressure and prevented any rise in diastolic pressure

They concluded that at least up to heights of 14,000 feet, the inhalation of CO_2 was beneficial in protecting them against strain on the heart and respiration from acapnia and anoxia during physical exertion.

On the other hand Johnson and his co-workers (128) found that with dogs at pressures equivalent to 35,000 feet there was the same fall in the oxygen saturation of venous blood whether they were inhaling 100 per cent O_2 or a mixture of 91 per cent O_2 and 9 per cent CO_2. They concluded that there were no benefits from the added CO_2, but in view of the fact that there was no greater fall in O_2 saturation with added CO_2, one might expect the increase in cerebral blood flow itself to be beneficial.

In men they found that there were less adverse subjective effects if 2 to 3 per cent CO_2 were added to the inhaled O_2, but since there was no rise in alveolar O_2 pressure on hyperventilation with the mixture, they concluded that there were no benefits from adding CO_2 to the inspired air at high altitudes but, again, one would expect the increased cerebral blood flow to be beneficial and this probably accounted for the improved subjective feelings. In Barach's (129) opinion the adverse effect due to the loss of 10 per cent oxygen when it is replaced by 10 per cent CO_2 is too great to be offset by any beneficial effects of the CO_2 and less than 10 per cent CO_2 would be ineffective at high altitudes (40,000 feet).

It is certainly desirable to have quantitative work on this exact problem of how much oxygen can be safely sacrificed in order to substitute carbon dioxide in such a way that the advantages of the latter outweigh the adverse effects of lessened oxygen supply.

Beyne and Bergeret (130) calculate that sufficient CO_2 must be added to the inhaled O_2 to raise the partial pressure of the alveolar air to 40 mm. Hg at any given altitude; i.e., the alveolar CO_2 pressure must be kept at the normal sea level value. To achieve this result at an altitude of 30,000 feet they found that 8 per cent CO_2 with 92 per cent O_2 was effective. They designed a mask with a reservoir to trap some of the expired air so that a certain amount of this could be rebreathed. At an altitude of 30,000 feet a reservoir of 800 cc. in the mask supplied sufficient CO_2 to maintain the alveolar CO_2 pressure at 40 mm. Hg.

Although there is great individual variation in the response to CO_2 administration during oxygen-lack (131), it is generally recognized that its effects are of real importance in the maintenance of normal function at high altitudes (Heim (132)). The practical advantages, in fact, seem to be so great that some air lines have considered releasing CO_2 in their cabins at high altitudes (133).

The physiological details of the alleviating effects of CO_2 in anoxia have for the most part been worked out by Gellhorn. He has shown (134) that with gas mixtures containing 8.5 per cent O_2, the fall in systolic blood pressure which is such a danger to the cerebral circulation can be more or less completely prevented by the addition of 3 per cent CO_2 to the inhaled mixture. The same conclusion was reached independently by Denian (135). Gellhorn believes that the CO_2 restores the reflexes of the carotid sinus centers which have been suppressed by O_2 want.

Gellhorn also found (120) that the changes in visual intensity discrimination due to oxygen-want (produced by inhaling 8 to 9 per cent O_2) can be completely

avoided by adding 3 per cent CO_2 and disturbances in handwriting are also eliminated (136). Spiesman and Gellhorn (137, 138) found less rise in the auditory threshold when 4 to 7 per cent CO_2 was given during anoxia, i.e., the decrease in excitability of the sensory cortex due to anoxia is not so extreme when CO_2 is added to the inspired air.

It is of importance that the eye functions, found by Gellhorn (139) to be impaired by low oxygen, all involve cortical function (e.g., visual acuity, intensity of discrimination (140), color perception, formation of negative after-images (141), and extent of visual field). In contrast, the reflex eye movements which do not involve the cortex (e.g. nystagmus (142, 143)) were not affected but even enhanced by anoxia. This result bears out Kabat's observation that the cortex of the brain and cerebellum is more sensitive to anoxia than are subcortical structures (144). It also confirms Gellhorn's observation (145) that, whereas anoxia causes a decrease in the reactivity of the cortical, subcortical and spinal centers (146), it results in an increased reactivity and increased tonic discharge of autonomically innervated centers.

Although the increase in pH due to the hypernea of anoxia tends to increase the nervous excitability in general, it does not have this effect on the cortex because it is more than counterbalanced by the reduction in blood supply to the brain (Gellhorn and Joslyn (147)). Addition of CO_2 to the inspired mixture helps to circumvent this by dilation of the cerebral blood vessels.

Clarke (148) in pressure chamber experiments on men, found that the addition of 8 per cent CO_2 to the inspired air kept the alveolar CO_2 level constant up to a simulated height of 20,000 feet in spite of a 77 per cent increase in the volume of breathing, whereas with air alone there was a 23 per cent drop in alveolar CO_2. The added CO_2 had very little effect on the alveolar O_2 tension, and none on the symptoms of anoxemia, but it should be remarked that in these experiments CO_2 was added to air and not to oxygen in the inspired gas mixture.

Gellhorn has taken these studies further, i.e., into the field of mental tests where essentially the same effects of CO_2 were demonstrated, in that the circulatory changes due to the added CO_2 lessened the impairment due to anoxia (147, 149, 150) (151). A similar conclusion was reached by Loevenhart (152) in experiments on the therapeutic use of CO_2 in mental diseases.

There is another aspect of aviation in which the use of CO_2 has been advocated, and this is in counteracting the effects of the circulatory changes due to sudden deceleration of the plane. Kleinschmidt (153) studied these changes and advised the use of CO_2 in a concentration of 7 per cent. His views are supported by Ruff and Stroghold (154) and by Matthes (155). Livingston (156) found that O_2 has little effect in holding back the onset of "black out," whereas CO_2 appeared to be beneficial and suggested that this might be due to stimulation by CO_2 of adrenaline output.

THE PROBLEM OF CO_2 ADMINISTRATION IN CONDITIONS OF EXTREME COLD

The maintenance of body temperature is still one of the major problems in high altitude flying. Cockpit heating is clearly no solution in fighter planes where the cabin may be pierced by bullets; the same may be said against the use

of compressed air cabins in military aviation. There is not only the factor of lowered external temperature to contend with at high altitudes, but also the fact that anoxia itself results in a loss of body temperature (157, 158, 159).

Even with simulated altitudes in a pressure chamber Behague (160) was able to demonstrate a fall in body temperature in rabbits at 22,500 feet. Gellhorn and Janus (158) have proved this fall in body temperature to be due to the lower partial pressure of oxygen and not to the lowered barometric pressure. There is also an intensification of pilomotor reactions in anoxia (138).

The loss of body temperature in anoxia is not relieved by inhalation of 3 per cent CO_2 but is in fact increased. This is not principally due to increased metabolism, or to heat loss from the lungs during the hypernea caused by the CO_2, but is chiefly due to peripheral vasodilation (161, 162, 163). The addition of 3 per cent CO_2 to the inhaled air does not alleviate this as the resultant increase in oxidation in the tissues is not sufficient to counteract the heat loss from vasodilation (Gellhorn (159)). Subjectively, however, there is a feeling of warmth (134).

In open cockpit flying, wind has a slightly favoring effect through an increase in pulmonary ventilation (Schneider (164)); this is the reason why there is a slightly higher "ceiling of resistance" in open planes than in simulated altitudes in the pressure chamber. A wind of 25 miles per hour is sufficient to cause this increase in pulmonary ventilation (165). Even a breeze of only 15 miles per hour at a temperature of around 60°F. will increase the metabolism by 19 per cent; this increased demand for oxygen is the result of the cooling effect of the breeze (166).

The excessively low temperatures encountered at high altitudes are due to the lowered molecular concentration which increases radiation but decreases convection and conduction of heat (Poppen (167)). Grow (168) has studied the factor of cold in high altitude flying and finds that it produces the same mental symptoms as anoxia. With cold there is a decrease in the O_2 utilization of the tissues, as shown by the arterial-venous O_2 tension difference and he suggests that the administration of O_2 with small amounts of CO_2 added may alleviate the results of extreme cold.

DISCUSSION

Study of the literature reveals the manifold role of carbon dioxide. Even when the issue is narrowed to its role in anoxia, effects all over the body come into play.

The greatest simplification of the problem—the influence of carbon dioxide upon anoxia—may perhaps be stated as follows:

Under the influence of anoxia the respiratory volume increases, and changes take place in the circulation. These regulatory processes which are evoked by anoxia are gradually weakened by the loss of carbon dioxide which accompanies the increased respiratory ventilation (Gellhorn (169)). The addition of small quantities of CO_2 to the inspired oxygen combats this effect and greatly improves the respiration (133).

The mechanisms by which CO_2 combats this effect of anoxia are many:

a) Increase in respiratory volume due to direct stimulation of the respiratory centers.

b) Shift in the oxygen dissociation curve of the blood, so that O_2 is released more rapidly to the tissues.

c) More rapid blood flow through the brain caused by: the direct effect of carbon dioxide on the cerebral blood vessels; by the increased sensitivity of the vasomotor centers; by reflex stimulation of the carotid and aortic bodies; and also by augmentation of the rise in blood pressure caused by anoxia (134, 34).

d) Improved venous return caused by the venopressor effect of CO_2 (Henderson (170)) which combats the loss of muscle tonus due to anoxia (169, 171) and by the direct mechanical effects on the veins of increased respiratory pumping.

The beneficial effects of CO_2 in oxygen deficiency are thus not inconsiderable; the application of this knowledge to the problems of oxygen deficiency in high altitude flying is one which can only be determined by laboratory experiments on man.

It would be desirable to set up critical experiments with lowered O_2 tensions in which subjects were taken down through the intermediate confused states which lie between consciousness and complete unconsciousness. A carefully designed series of tests could be applied to determine the progression of motor and sensory impairment, coordination loss and mental confusion, and such objective observations as can be made by the electroencephalograph and by ophthalomoscopic observation of the retinal vessels should be run concurrently. The oxygen threshold for various stages of deterioration in an individual should be determined both with and without inspired CO_2.

It is essential to establish whether and to what extent O_2 tension in the arterial blood can be sacrificed in order to gain the benefit of increased blood flow through the brain, since this is perhaps the most important mechanism of its beneficial effects. The final decision as to the value of carbon dioxide in problems of high altitude flying should be based not on its effect on arterial oxygen tension or on any other single functional activity, but on the actual performance of the individual.

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TONUS AND THE VENOPRESSOR MECHANISM: THE CLINICAL PHYSIOLOGY OF A MAJOR MODE OF DEATH

YANDELL HENDERSON, PH D., M D. HON.

"The greater part of the skeletal musculature is all the time steadily active"

—*C. S. Sherrington, 1904.*

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I. CLINICAL PHYSIOLOGY

In the year 1911 the American Medical Association held its Annual Meeting in Los Angeles In the Section of Pathology and Physiology it fell to me to deliver the Chairman's Address. I chose as my title "Clinical Physiology—an Opportunity and a Duty." I said: "Great is the medicine of today in its knowledge of man's parasites; great in its acquaintance with the appearance of his tissues after he is dead. But do we not lack an adequate comprehension of the functions of the man himself, and a control of the working of the machine while, although somewhat damaged, it is still a 'going concern'?"

Clinical medicine is now far more receptive of physiology than it was 30 years ago, but there is still far too little clinical physiology for it to receive and to apply. On no topic is it more deficient in fundamental understanding and on none could physiology make a more useful contribution than on: "How men die"

This article summarizes my studies on that subject during the past 40 years. The data upon which it is based are presented in detail in my "Adventures in Respiration: Modes of Asphyxiation and Methods of Resuscitation"; and with H. W. Haggard in "Noxious Gases and the Principles of Respiration Influencing their Action"

The line along which those "adventures" have led, and the degree of acceptance that they have attained up to the present writing cannot be better expressed and evaluated than they have been by Krogh as follows:

"For many years the circulation was regarded as if the heart action and the vasomotor (arterio motor) mechanism were the only variable factors, and as if any failure or depression not due to the former must be due to the latter. Observations by Yandell Henderson (1908) upon an experimental form of shock first demonstrated clearly the occurrence of what he termed failure of the 'veno-pressor mechanism,' characterized by decreased and finally inadequate venous return to the right heart, resulting in a decrease in cardiac filling and discharge, while the arteries at the same time were found to be, not relaxed, but constricted. Other papers by Henderson and his collaborators (1909, 1910) brought forward evidence in

favor of the view that it is this mode of circulatory failure rather than 'vasomotor failure' which is characteristic of shock and although there are a number of matters still needing further elucidation, this conception has now come to be generally accepted."

It is mainly to the "matters still needing further elucidation" that this article is devoted: in particular to the tonic booster pumps in the skeletal muscles that in health send the blood through the veins back to the heart: a factor in the circulation almost as important as the heart itself. It is this venopressor mechanism, and not the heart itself, nor the vasomotor system as conventionally conceived, that in a large percentage of all deaths is the factor that really fails and thereby brings the circulation to its final and fatal standstill.

II. TONUS AS THE INDEX OF VITALITY

It has been said that "the first business of the physician is to keep the patient alive long enough for him to get well." Facing a case of dangerous illness or injury, the physician's first step must then be to estimate the patient's vitality: whether it will increase and he will live, or whether it will decrease and he will die. Charles Darwin, who seems to have inherited much of his extraordinary powers of observation from his father, a physician, described him as quite unscientific, yet as carrying on a wide practice successfully largely because "his power of predicting the end of an illness was unparalleled."

Upon what, 150 years ago, could Dr. Darwin or any other physician have based accurate prognoses? Or, for that matter, even today are not prognoses on cases of extreme prostration after severe wounds or illness, more observational than scientific?

If, then, the art of foretelling life or death is to be developed into a science, so that it may be practiced accurately even by those who lack the Darwinian supergift of observation and pictorial memory, it is essential to determine first: What is vitality? In what does failing vitality consist? What are its indices? How may they be measured? And the answer, to be here set forth, is that to a very large extent vitality is equivalent to a certain master function of the body—a function that is now only vaguely appreciated in clinical medicine—the function of tonus.

Roughly, tonus, as that term will be used here, is the peculiar vital property of the muscular tissues induced and controlled in those tissues by the motor centers in the central nervous system. Thus tonus, although manifested in the musculature of the body, has its seat and control in the grey matter of the spinal cord and bulb and is exerted upon the muscles—or reflected back and forth between centers and muscles—through the fibers afferent and efferent in the motor nerves.

Nothing about the body is more obvious than that it moves and does external work; and that the motions and work are effected by contractions—actual shortenings—of the muscles. On the other hand, nothing is less obvious, at least to superficial observation, than that almost the entire musculature of a healthy man, when lying as completely relaxed as possible, is nevertheless continually exerting innumerable elastic pulls. As the genius of Sherrington first expressed it, "The skeletal musculature is all the time steadily active" in tonus.

These two activities of the muscles—motility and tonus—although largely distinct in purpose, are nevertheless both operated through the same nervous and anatomical machinery. Each is essential to life and health: tonus no less—indeed rather more—than motility. Life could continue for long with almost no movement. Without tonus, it would cease immediately. Indeed, the death of the body is almost the same thing as abolition of tonus.

More closely defined, tonus consists in a peculiar vital form of muscular elasticity. The human frame is a structure that is anatomically and gravitationally wholly unstable. Yet by the steadily maintained, elastic pulls of tonic muscles, it is enabled to stand erect, to hold up the head for hours on end, and—with a minimum of fatigue—to assume and maintain innumerable different postures. As a form of elasticity, tonus far exceeds the pull or push of rubber or of any spring. By this capacity, each muscle may be adjusted to many different lengths—some long, some short—as needed for each particular posture; and at each length it may for long periods exert just that degree of elastic pull that will hold the bones to which it is attached at just the angle needed for the posture. It is also largely through the tonus of the skeletal muscles during bodily rest that the basal energy expenditure of the body is maintained: together with its concomitant heat production, oxygen consumption, and carbon dioxide production.

Without tonus the trunk, the limbs, and the face—as seen in facial paralysis—are as limp and sagging as a wet cloth. The chest loses the greater part of its volume, as the diaphragm bellies into the thoracic cavity: allowing the lungs to collapse to an air capacity less than that in the deepest voluntary expiration, and rendering breathing impossible. Such is the condition in the baby that at birth fails to develop sufficient tonus to inflate its lungs—at least partially—and therefore can never breathe.

Without the tonic activity of the skeletal musculature the circulation of the blood also ceases; for even if the heart continues for a time to contract and relax, the blood stagnates in the atonic tissues; and the heart can pump into the arterial system only such a volume of blood as the venous return brings to it. This is true of the newborn also. Neither respiration nor circulation can be established without the support of the tonus of the musculature of the infant's body; nor maintained without it in an adult.

Of all of this, of course, Dr. Darwin knew next to nothing. Yet he was evidently an extraordinarily able diagnostician; and he probably used such therapy as was then available with a skill that was equally based on his instinctive power of observation and wide experience. He did not know what tonus is: no one then did. Yet for many an ailing patient he prescribed a "tonic t.i.d.": one or another of the multiple concoctions that have long since been discarded and forgotten. The symptoms that he observed were nonetheless the signs of varying degrees of tonus, or its depression. Like every physician or surgeon, before and since, if at all possessing the naturalist's gift of observation, he estimated in every serious case of illness or injury the degree of vitality from these signs and symptoms. He saw that at the lower end of the scale of vitality the patient is so weak that he cannot lift his head off the pillow or speak above a whisper. In the middle of the scale he saw that the elderly man in poor health no longer walks

with a springy step: The "extensor thrust" reflex is feeble. He saw that nothing so immediately decreases the signs of tonus as does the inhibiting influence of pain: a painfully wounded man cannot stand. And, among his children and many grandchildren and others, he saw that the healthy child skips and bounds —by reflex muscular contractions of the "knee-jerk" type—as if its muscles were rubber bands or steel springs, as all children do when well; and do not, if their tonus is depressed even by a cold.

Yet, while few clinicians will doubt that these signs of apparent elasticity, or its depression, are largely expressions of the degree of tonus, still fewer as yet think—or at least speak and write—of this scale of health and illness in terms of tonus. And when they do use the word "tonus," they usually have in mind the tonus of the blood vessels under the control of the vasomotor center in the medulla oblongata, which is something quite different. It is not arterial pressure, but the tonic influence of the motor centers in the spinal cord upon the skeletal musculature which is the factor that is really mainly involved in the tonus of the body. It is that factor also, and not vasomotor tonus, that determines the volume of the venous return, and so also of the circulation.

Among the functions and conditions determined by the tonic nervous discharges of the spinal centers which are to be discussed in the following sections—functions and conditions which Dr. Darwin could not, but the physician of today can, measure as indications of vitality—are:

(1) The resting oxygen consumption of the body.
(2) The resting heat production.
(3) The knee jerk and similar reflexes.
(4) The intramuscular pressures that tend to induce the return of blood to the heart.
(5) The electromyogram indicating the activity of the intramuscular booster pumps.
(6) The volume of the venous return as shown by measurement of the height of the column of blood in the veins of an arm held vertically when the body is in the head down position.

And now before taking up the details of the failure of all of these functions and conditions when tonus fails, we may call to mind the classic description of death by loss of tonus. It was written by an even more brilliant observer of human nature than either of the Darwins. He was quite without medical science. He put the words in the mouth of Mistress Pistol, née Quickly.

"Nay, sure, he's not in hell: he's in Arthur's bosom, if ever man went to Arthur's bosom. A' made a finer end, and went away an it had been any christom child; a' parted even just between twelve and one, even at the turning o' the tide: for after I saw him fumble with the sheets and play with flowers and smile upon his fingers' ends, I knew there was but one way; for his nose was as sharp as a pen, and a' babbled of green fields. 'How now, Sir John,' quoth I: 'what, man! be o' good cheer.' So a' cried out 'God, God, God!' three or four times. Now I, to comfort him, bid him a' should not think of God; I hoped there was no need to trouble himself with any such thoughts yet. So a' bade me lay more clothes on his feet: I put my hand into the bed and felt them, and they were as cold as any stone; then I felt to his knees, and they were as cold as any stone, and so upward and upward, and all was as cold as any stone."

Shakespeare published those words in 1623 Five years later, 1628, Harvey published "De Motu Cordis" In it he not only showed how the heart beats, but also gave the first indication of how and why body heat, the venous return, the normal appearance of the nose and other features failed in old Sir John Falstaff with the failure of tonus

This condition and mode of death—alike after illness and trauma—are now commonly called "peripheral circulatory failure" and "shock" I would suggest instead the terms "hypotonia" and "tonic failure."

III MUSCLE TONUS AND THE VENOUS RETURN

"We have yet to explain in what manner the blood finds its way back to the heart from the extremities by the veins" So wrote William Harvey: and now, 300 years later, the explanation is still not wholly complete As a major factor in the venous return, Harvey described the valves in the veins and showed, by moving a finger along a vein in the arm, that "while these valves readily open in the right direction," i e, toward the heart, they "entirely prevent all contrary motion" And he accompanied the description with a drawing, copied from Fabricius, showing a man's forearm with a ligature above the elbow and the hand grasping a rod, while the veins swell. In the grip of the hand in that drawing is the first suggestion of a venopressor mechanism, and its dependence on muscular contraction, and so also on muscle tonus

It is always dangerous to read subsequent knowledge back into the words of the first author in any field Yet one cannot resist the impression that Harvey, in this drawing along with his account of the valves in the veins, recognized that the vigorous contraction of the muscles of the forearm propels blood from the muscles into the veins and on toward the right heart. If so, he would have been entirely in accord with the modern view that any muscle that is rhythmically relaxed and contracted, so that its capillaries are alternately filled from the arteries and emptied into the veins, acts as a peripheral pump, a "booster," aiding the venous return of the blood and the diastolic filling of the heart.

How well the intramuscular blood vessels are arranged to act the part of such peripheral blood pumps is clearly described by Krogh. He writes:

> "The arteries supplying a muscle branch freely and between the branches there are very numerous anastomoses forming a primary net The capillaries unite into venules intercalated between the arterioles, and the whole system of veins reproduces and follows almost exactly that of the arteries All the veins down to the smallest branches are provided with valves allowing the blood to flow in the direction of the heart only When the muscle contracts its form is greatly altered, the fibers become much shorter and proportionally thicker, . The blood is driven out by compression from a number of the venous branches and, when the muscle relaxes again, these can be filled from the peripheral end only. Since muscular contractions usually more or less regularly alternate with relaxations the system of valves makes of the veins of each muscle a very effective pump ." (For his investigations in this field Krogh was awarded a Nobel prize)

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aid to the venous return that the enormous increase in the output of the heart—at least five fold—during vigorous muscular exertion is made possible.

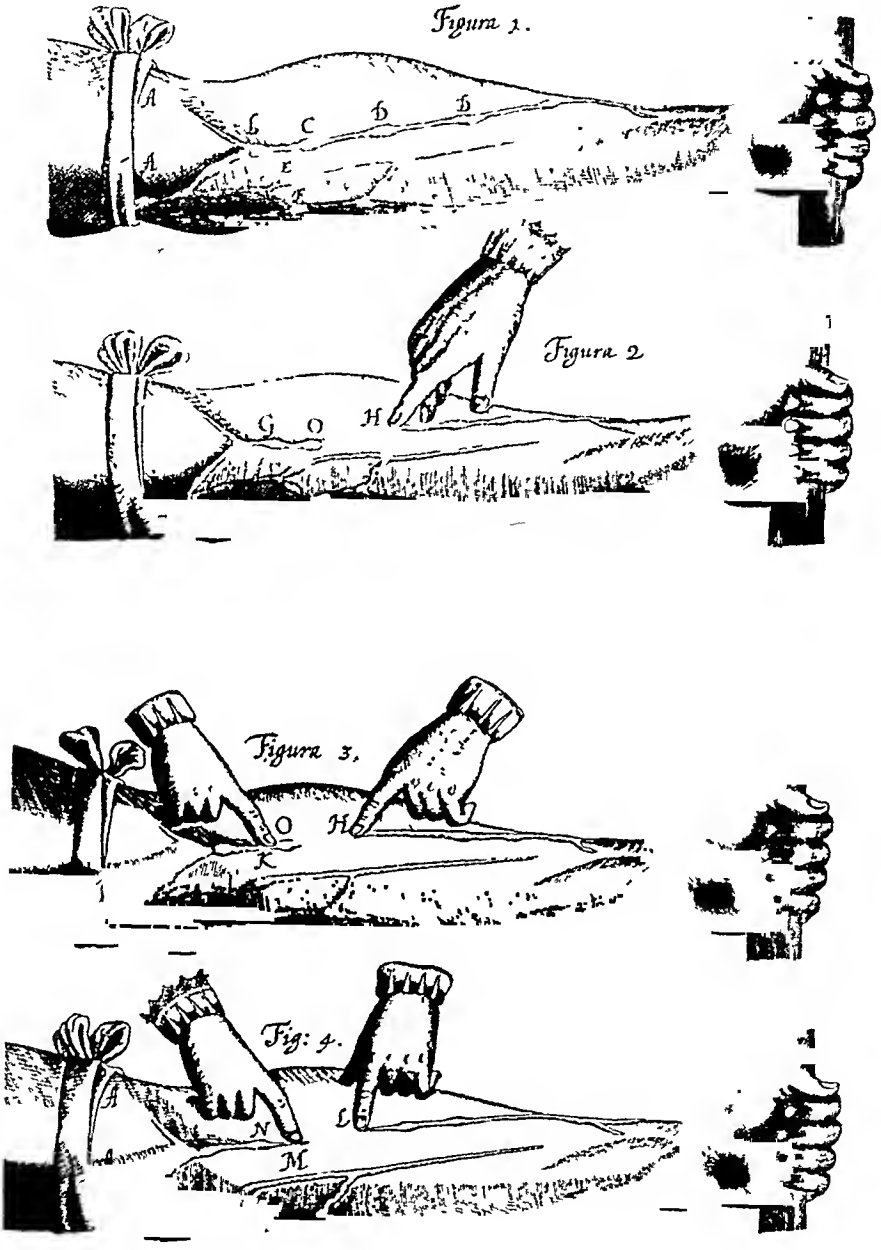


FIG. 1. THE DRAWING USED BY HARVEY, AFTER FABRICIUS, TO SHOW THE VALVES IN THE VEINS

Note the grip of the hand, the contraction of the muscles of the forearm and the swelling of the veins

But suppose the exertion ceases and the man stands quite still, or sits down, or lies down and rests so completely that for a time no muscle in his body makes any visible movement, or does any external work. Does the pumping action

of the muscles cease entirely? Is the venous return then wholly dependent upon the vis-a-tergo imparted by the heart and transmitted through the arteries and capillaries into the veins, and so onward to fill and distend the right heart? In other words, to paraphrase Harvey's question, "could the blood find its way back to the heart from the extremities by the veins" without some peripheral aid? There is strong evidence that it does not, and that it could not; but that at least in health there is constantly active peripheral aid to the venous return. It is only in the state called shock and other conditions of profound physical depression and muscular relaxation that this booster action fails; and induces in turn the failure of the circulation that for so long has been erroneously ascribed to "vasomotor failure." The testimony now to be cited indicates that even in the most complete state of healthy rest and quiescence the muscles are still pumping and that the venopressor mechanism, as above described, is still active so long as the motor centers in the spinal cord continue to discharge nervous impulses into the skeletal muscles in the maintenance of tonus.

Sherrington, in his classic analysis of the proprioceptive reflexes by which muscle tonus, body posture and facial expression—and to a considerable extent also basal metabolism—are maintained and adjusted, showed decisively that even when the body is at rest, and appears to be entirely motionless, the skeletal musculature is far from flaccid. On the contrary, as he expressed it, "the greater part of the skeletal musculature is all the time steadily active."

More recent studies, such as those of Adrian and Adrian and Bronk on single nerve fibers, have confirmed the conception that the mode of behavior of all the nerve-muscle mechanisms involved in external motion and active work holds equally true of the tonic contractions of the muscles that maintain posture even in normal rest. Between the states of bodily exertion and muscular rest, apparently so different, the form of activity within the muscles is exactly the same: varying only in degree.

In the so-called "isometric contractions," that tonus maintains when a man is standing or sitting, and even when lying down, the muscles do not shorten and do no external work. They do, however, exert an elastic pull to maintain posture. A weak pull of this type, which for lack of a better term is here called tonus, is due to impulses from motor centers in the spinal cord over only a few nerve fibers which induce contractions only in correspondingly few bundles of muscle fibers; but the impulses over each of these nerve fibers and the contractions, or elastic pulls, of each of the bundles so stimulated are maximal. A stronger pull of the muscle is due to impulses over a greater number of nerve fibers and a more rapid succession of impulses in each fiber, which stimulate a greater number of bundles of muscle fibers: the bundles contracting, or pulling, not all together, but in relays and rotation.

Contrariwise, as the activity of a muscle decreases to lower and lower degrees of intensity, until it is apparently wholly quiescent and doing no external work whatever, nervous impulses pass over fewer and fewer nerve fibers from the motor centers; and fewer and fewer groups of muscle fibers are at any instant active. Yet it appears probable that no living muscle is ever allowed to cease its tonic pull completely. Always a few of the motor nerve fibers to it are discharg-

ing impulses into it, and a few of its bundles of muscle fibers are stimulated to contract.

Such, as nearly as the picture can be constructed from the experimental data now available, is the state of continual internal activity which is skeletal muscle tonus. It is a state which no part of the body loses completely except at death. So long as it is maintained, it involves continuance of the pumping action of the discrete muscle bundles relaxing and contracting one after the other and thereby filling, compressing and emptying their intercalated capillaries and veins in the manner that Krogh has described for the muscle as a whole.

The action of these minute pumps is the principal, but not the only, aid that muscle tonus affords to the venous return. A longitudinal pull on such a structure as a muscle necessarily induces a general internal pressure; and a pressure anywhere in a system permeated with collapsible vessels containing a fluid tends to cause the fluid to flow toward any point where the pressure is lower. That is the basic conception of hydraulics and hemodynamics. If, then, the state of tonus in muscles maintains an intramuscular pressure higher than that of the atmosphere about the body, and the pressure in the thorax is subatmospheric—the two pressures together constituting the effective venous pressure—the tonic intramuscular pressure must aid the venous return that distends the right ventricle. That such intratissue pressures do normally exist is now demonstrated by a considerable literature. They are measured by determining the pressures required to force a minute amount of a saline solution through a hypodermic needle into a muscle or other tissue; and the pressures so demonstrated are found to vary according to the tension on the muscles: that is, their tonus. By maintaining pressures upon other tissues, as in the abdomen, muscle tonus conserves in the capillary blood some of the energy that the heart has imparted and tends to press onward toward the veins the blood which the arterioles throw into them.

The Electromyogram

Every time that a muscle fiber contracts it manifests its activity by an electrical event, the so-called action current. If, then, a muscle such as the biceps in a man's arm containing hundreds of separate muscle bundles is maintained in a state of tonus, the number of bundles that are thrown into contraction at any instant should be revealed in the electromyogram. In other words, the number of electrical impulses appearing in such a graphic record should indicate the number of minute capillary and venule pumps that are making discharges toward the heart. If this is correct, a series of such electromyographic records made at times when the biceps is entirely immobile externally, but under different degrees of tension, should show varying degrees of electrical activity corresponding to the degrees of tension. Accordingly, at my request, my colleagues of the Section of Neuro-Anatomy have recorded the series of electromyograms which is here reproduced. They reveal that the biceps of a man lying at rest with his arm relaxed, as in the first record (B), or perfectly quiescent under slight strain, as in the other records, is "all the time steadily active." They show that the number of bundles of muscle fibers in a muscle that are nervously activated

varies with the intensity of the longitudinal pull that the muscle maintains. And the same evidence, interpreted in accord with Krogh's description, indicates that the number of minute pumps that are actively aiding the venous return to the heart varies correspondingly.

So at last in the activity of the peripheral booster pumps, and the intratissue pressure, maintained throughout the body by muscular tonus, we find the answer to Harvey's question "in what manner the blood finds its way back to the heart from the extremities by the veins" And we find also how by failure of the venous return—as all investigators now agree—rather than by failure of the heart itself or vasomotor failure, a large percentage of all men die.

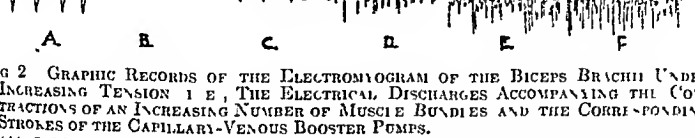


FIG 2 GRAPHIC RECORDS OF THE ELECTROMYOGRAM OF THE BICEPS BRACHII UNDER INCREASING TENSION I E, THE ELECTRICAL DISCHARGES ACCOMPANYING THE CONTRACTIONS OF AN INCREASING NUMBER OF MUSCLE BUNDLES AND THE CORRESPONDING STROKES OF THE CAPILLARY-VENOUS BOOSTER PUMPS.

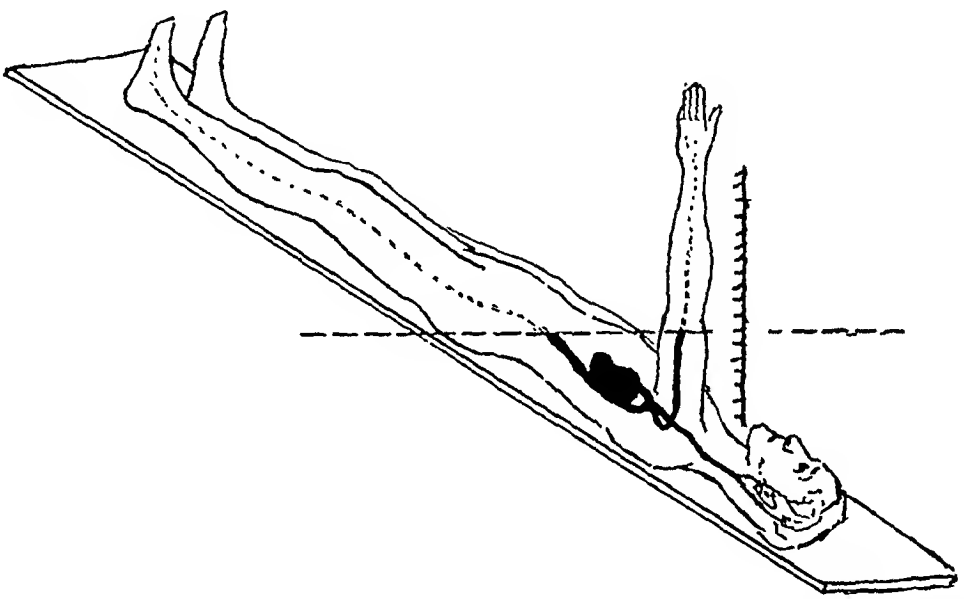
(A) Calibration, 50 micro volts. (B) Subject lying supine, arm beside body, biceps "at rest" (C) Biceps contracted sufficiently to hold the elbow flexed at 15 degrees (D) Same position, 300 g weight in hand (E) Same position, 600 g weight in hand (F) Same position, 1200 g weight in hand Arm perfectly quiescent during the taking of each record Records by courtesy of the Section on Neuro-Anatomy (Graph by L F. Nims)

IV. ESTIMATION OF THE VENOUS RETURN

Before the venopressor mechanism can play any considerable part in clinical thought, it is essential that the variations in the volume of the venous return shall be made so evident by some form of measurement that they can be appreciated quantitatively. Arterial pressure—unwisely called "blood pressure"—is seen and appreciated quantitatively because it is measured with the sphygmomometer. It is equally important that the venous return shall also be made evident and quantitative. This object—it should be emphasized—cannot be attained by measurements of venous pressure with the patient in the standing, sitting or recumbent positions Under just those conditions of failing vitality in which it is most important for the clinician to follow the decrease or recovery of the venous return, venous pressure is immeasurably low. Fortunately there is a simple method by which the venous return can be accurately estimated, and its variations expressed in figures. For this purpose it is only necessary to invert the body to such a degree that all the blood returning from the tissues is—so to speak—poured into the great veins near the heart: the preventricular reservoir of von Recklinghausen. There the volume so collected can be estimated by measuring the height of the column of blood in the veins of an arm held vertically, or lifted gradually, until at a definite elevation a part of a vein collapses.

The measurement may also be made, more elaborately, but no more significantly, by means of a needle in the arm vein and a manometer, as in the ordinary venous pressure method.

The significance of observations made under such conditions will perhaps be most easily shown, if the manner in which the technic came to be developed is first described. It was as follows: Early in 1917, Haggard and I carried out a study of the circulation in healthy men held comfortably in a nearly upside down position. The apparatus which we used consisted of a heavy plank hinged on two uprights near its middle with an adjustable footrest at one end and two iron hooks to hold the shoulders at the other end. The subject first stood upon the footrest and leaned against the plank which was then turned to the horizontal with the subject lying upon it, and then further to a position in which the subject was inverted to any desired angle—usually 45° from the vertical—and fixed in



. 3. The Preventricular Reservoir of a Man in the Head-down Position (1:4) Showing how the height of the column of blood in a vein of an uplifted arm may be used estimating the volume of the venous return and the need for infusion of blood or serum.

. .t position. Any angle beyond 45° was found to throw the weight of the body upon the shoulders and against the hooks to an extent involving strain and some discomfort. At 45°, or a slope of 1:1, or at any lesser angle and slope, the subject after one or two experiences was usually quite comfortable and could remain for several minutes at a time without complaint. The observations upon arterial pressure, pulse rate and heart sounds made under these conditions confirmed those of Leonard Hill upon animals and may be found in our paper. They show that the head down position is not so much of an aid to the circulation as surgeons generally believe.

That position may, however, be of great importance for diagnosis. For when one of the subject's arms was held vertically, or lifted gradually, the top of the column of blood in the veins showed a sharply defined meniscus and the height of that meniscus above some point of reference, such as the symphysis of the clavicles, could be quite accurately measured in centimeters. After a few min-

utes of muscular exercise, such as stair climbing, the height of the column was markedly increased, as it was also by inhalation of a moderate amount of carbon dioxide. Contrariwise after forced breathing, a lowering of the column accompanied and followed the period of apnea.

Evidently here was a method that, if the permission of our surgical colleagues could be obtained, might be applied clinically. Already for more than ten years, in fact from 1906 or 1907 to 1917, we had noticed in this laboratory that in all animals, particularly dogs in normal condition and lying on their backs before

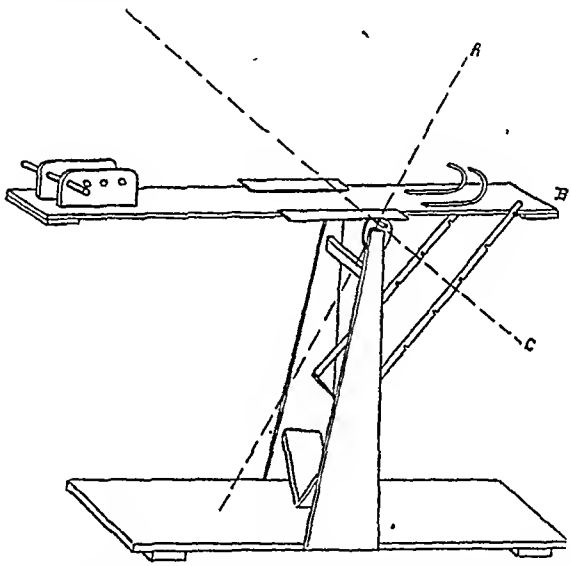


FIG. 4. THE TILTING BOARD USED TO TURN A PATIENT INTO THE HEAD-DOWN POSITION FOR MEASUREMENT OF THE HEIGHT OF THE COLUMN OF BLOOD IN AN ARM

With the board in position A, the patient stands on the footrest and leans against the board which is then turned first to position B horizontal and then to position C inverted.

experiments—as is true also of healthy men—the jugular veins were well filled, but that after an hour or more under anesthesia and operation, usually in the abdomen, the jugular veins were no longer well distended. They were, on the contrary, empty and collapsed: the venous return had greatly diminished. We were therefore anxious to learn whether a similar indication of lowered vitality and diminished venous return occurred in major surgical operations on patients. To this end fortune favored us, and no surgeon's permission was needed. In the New Haven Hospital at that time there was in the passageway leading to the operating room a part, or ramp, 8 or 10 feet long where the floor sloped at a grade of slightly more than 1 in 4. All patients were wheeled down this slope on

a stretcher before operation and up it afterward. It occurred to Haggard, then a student substituting for an interne, to have the stretcher turned so that the patient's head was downward, and while he was in this position for a few seconds to measure the height up to which the veins in the elbow were full and above which they were collapsed. Similar measurements after the operations indicated the amount of depression of vitality and diminution of venous return induced by the anesthesia and operation. For major surgery of that time (1917) the decrease was commonly from 6 to 9 cm. Recent observations indicate that under the better anesthesia and gentler operative technic of today the decrease of venous return is usually less.

A few observations were made also on patients with pneumonia or typhoid fever. The foot of the bed was lifted on to a wooden horse 60 cm. in height, giving a slope of about 1 in 3.1. Care was taken that at every observation the patient's head was just touching the head of the bed. The height above the floor at which the chosen vein collapsed could then be accurately determined and the changes from day to day in relation to the course of the disease recorded. Acute illness was generally correlated with a markedly low venous column, which fell progressively further as death approached. Then as the body lost all tonus, the column reached zero on this index of tonus and manometer of vitality.

Such measurements of the venous return have recently assumed a greatly increased practical importance. In failure of the circulation, due either to muscular atonicity after severe injury or to hemorrhage, the provision of dried serum, rediluted, makes immediate infusion possible. What has long been needed is some simple method of estimating when, and in what volume, serum should be given. Then the surgeon need not wait until the patient's skin is drained of blood and his "nose as sharp as a pen." Particularly after burns, if the loss of serum externally is not to induce a development of shock, immediate and repeated restoration of the fluid of the blood is imperative. And for this purpose estimation of the decreasing venous return is a more logical and direct means than measurements of arterial pressure with a sphygmomanometer, and far more significant than the ordinary measurements of venous pressure in the recumbent position.

V. MUSCLE TONUS AND RESPIRATION

Skeletal muscles have little intrinsic activity. Unlike cardiac muscle their behavior is imposed upon them from the central nervous system. To trace the various influences that act upon the accessory blood pumps in the muscles, we must therefore look behind the muscles to the motor centers in the spinal cord which innervate them. Yet it is only by the behavior of the muscles that we can judge how various influences affect the state of their motor and tonic centers.

As the means of testing that state two types of experiments are available. One is by means of what Sherrington has termed "proprioceptor reflexes," of which the most convenient is the knee jerk. For this purpose the thigh must be supported and fixed in an unchanging position and the taps on the patella tendon must be applied at uniform intervals by a light hammer falling through a uniform

distance. With this arrangement it has been found that virtually any and all events anywhere in the body affect the knee jerk either to an increase or decrease; inspiration, expiration, clenching a hand, mental arithmetic, somnolence, a sudden loud sound, and so on almost endlessly are such influences. In the present connection, the most significant influence upon the motor centers is, however, chemical: the influence of the blood gases. This influence was clearly demonstrated in experiments performed with the collaboration of Coffey and Barnum, in 1909, in which we found that a period of forced overbreathing sufficient to induce a slight apnpnia (deficiency of carbon dioxide in the blood) and a subsequent brief period of failure of breathing, was followed also, for a slightly longer time, by the abolition of the knee jerk also. Contrariwise rebreathing from a small bag, until enough carbon dioxide had accumulated in the blood to induce a slight increase in the volume of breathing, induced a marked augmentation of the knee jerk. (For graphs of these effects, see Adventures in Respiration, page 241.) These facts suggest strongly that the blood gases, particularly carbon dioxide, exert an influence upon motor nerve centers that is manifested in muscle tension and the activity of the intramuscular booster pumps essentially as the blood gases are known to influence respiration. In other words, under ordinary normal conditions the amount of carbon dioxide produced in the body determines alike the volume of the air breathed, the volume of the venous return, and thereby the volume of blood circulated. In this way, as Miescher first recognized, the oxygen supply of the body is safeguarded by carbon dioxide.

One of the essential functional adjustments of the circulation is that by which in health just the right volume of blood is supplied to the right heart through the veins: not too much or too little. How this adjustment is controled was long obscure. Now it is clear: the amount of carbon dioxide produced in the tissues and carried by the blood to the central nervous system determines the tonic influences of the motor centers upon the muscles and so the activity of their booster pumps and the volume of the venous return. Alike in rest and during and after muscular exertion, the production of carbon dioxide determines in this way the volume of the circulation.

Now also it is clear how and why that "experimental form of shock" to which Krogh refers (as quoted in section I) results from overventilation through the causal sequence: (1) apnpnia; (2) depression of tonic nerve centers; (3) decrease of booster activity; and (4) failure of the venous return.

The other experimental method by which it is possible to test the state of motor centers in their control of muscle tonus has recently been employed by Henderson and Turner in their study of artificial respiration. They have shown that it is the "elasticity" of the thoracic muscles and diaphragm which makes artificial respiration possible; that this feature of these and other muscles is not a mere mechanical elasticity, but a vital property due to tonus under nervous control; and that in a normal man under artificial respiration it is the blood gases, acting upon the respiratory center and its subordinate motor centers, that determine the degree of tonic elasticity in the muscles that they innervate, and thus determine also the maximum volume of air that can be moved by

artificial respiration. More would be harmful by over-ventilation. These findings, which are in general accord with those of Hess in experiments on animals, are of particular interest in view of the campaign for the teaching of First Aid by the American Red Cross just now, as this teaching includes the Schafer prone pressure method of artificial respiration. Evidence both on animals and on healthy men in the laboratory, and in the resuscitation of cases of drowning, electric shock and carbon monoxide asphyxiation indicates that inhalation of a mixture of carbon dioxide and oxygen by increasing the tonic elasticity of the respiratory muscles is a valuable aid to manual artificial respiration in essentially the same way that it increases natural breathing. Both types of evidence show also that such devices as pulmotors, resuscitators, and other suck and blow apparatus are highly unphysiological. They impede the restoration of tonus; and sometimes damage the lungs. They take time to apply, and as tonus quickly fails in cases of asphyxia, drowning and electric shock, a delay of a few seconds often causes the loss of a life. (Note: On the contrary, in all the 45 years since the Schafer method was introduced, there is no record of its causing serious injury, such as a broken rib, in anyone.)

Such is the evidence obtained on normal men and on cases of asphyxia of various forms. It is easily checked and verified. Clinical experience shows correspondingly that during deep anesthesia, particularly for high abdominal operations in which extreme muscular relaxation is demanded by the surgeon, the tonus of the respiratory muscles is sometimes greatly lowered, the diaphragm is bellied up into the thorax and the volume of that cavity is correspondingly decreased. The partial collapse of the lungs so produced, is not alone sufficient to cause atelectasis; but it may permit plugs of mucus to accumulate in the narrowed airways and thus initiate that condition.

Atelactasis after Spinal Anesthesia

Most instructive are the occasional cases in which, under spinal anesthesia, the drug reaches the centers of the phrenics. The result is a temporary paralysis and complete relaxation of the diaphgram with extreme deflation of the lungs and, in some cases, plugging of one of the main bronchi. From a lung that is in any way occluded the gases are soon absorbed by a process that Coryllos first clearly showed to be entirely physical and to result in a massive collapse. For the reinflation of the lungs after the anesthesia and nerve block have worn off and the plug has been displaced, inhalation of carbon dioxide is now often used. It acts by increasing the tonus of the thoracic muscles and diaphragm and expanding the chest rather than merely by increasing the minute volume of breathing. If atelactasis is allowed to continue, it may lead to a secondary pneumonia.

Conversely, whenever a primary pneumonia involves the plugging of any of the airways, an atelactatic area inevitably develops in which infection flourishes. If, however, this area can be re-inflated, so that it again drains through its airways—as the lungs normally do—the infection clears up: at least such is the case in experiments on animals. The treatment of cases of carbon monox-

ide asphyxia and submersion with inhalation of carbon dioxide generally prevents both the atelectasis and pneumonia that were formerly common.

Alcoholic intoxication induces hypotonia and thus probably promotes the development of pneumonia in men who, in this state, are severely chilled.

Failure of the Circulation under Spinal Anesthesia

This general explanation of how temporary abolition of the tonus of the respiratory muscles may initiate atelectasis is closely paralleled by the explanation of how spinal anesthesia sometimes induces a temporary failure of the circulation. On this problem a clear description and conclusive analysis have been contributed by Schuberth and confirmed by Schneider. They have shown that the condition—virtually of shock—which not infrequently develops as a result of spinal anesthesia is due to the anesthetic reaching not merely the sensory roots, as intended, but also the motor centers and roots. Whenever motor nerves are blocked the tonus of the corresponding muscles is temporarily abolished. The paralysis of the muscles puts a stop to the activity of the booster pumps and lowers intramuscular pressure, with the result that the venous return from the area affected also fails. If a sufficient fraction of the total musculature of the body is affected, a general peripheral circulatory failure and death in shock may result. As everyone who has seen this condition has found, adrenalin is wholly ineffective: showing that "vasomotor failure" is not a factor in this form of shock. On the contrary, the vasomotor center is excited, and the arterial system is constricted in an attempt to compensate for the decreasing output of the heart and so maintain arterial pressure as long as possible.

VI. PERIPHERAL CIRCULATORY FAILURE AND ASPHYXIA

Whenever, as a consequence of acute illness or physical injury and pain, the motor centers in the nervous system cease to be "continually active" and fail in their normal tonic function, one of the accompanying manifestations is a failure also of the circulation. The nervous failure was formerly assumed to be primarily in the vasomotor centers, and to induce a relaxation of the peripheral blood vessels. To that conception the venopressor mechanism now affords an alternative. But no matter whether the primary failure is in the vasomotor control over the arteries and arterioles with "pooling in the splanchnic area," as heretofore believed, or whether, instead, the failure is in the spinal motor centers with loss of muscle tonus and slowing of the blood stream in the flaccid muscles, as here presented, the result is the same: a slowing of the blood stream.

Given a progressive slowing of the blood stream leading to peripheral circulatory failure and the first stage of shock, the next problem is that of how the second stage develops from it: that stage consisting in a decrease of blood volume which further diminishes the venous return and the minute-volume of the circulation.

In health, the blood stream is so large, or rather so rapid, that in each minute it brings to the tissues a large excess of oxygen; and the excess goes on into the

venous blood. When, however, in the development of traumatic shock or in the prostration of acute disease, the venopressor mechanism gradually fails, the venous return grows less and less, the circulation progressively slower and slower. Consequently the excess of oxygen brought to the tissues becomes smaller and smaller, until none is left to pass on into the venous blood. The arterial blood is still fully loaded with oxygen, but as the volume-flow decreases a point is finally reached at which the demands of the tissues are no longer met. Tissue asphyxia then develops, and with it a process analogous to edema. The walls of the capillaries become permeable and the serum of the blood and some corpuscles ooze out into the tissues. Three interacting results follow: oligemia, bradyrrhea and asphyxia: i.e., decrease of blood volume, slower and slower flow of blood through the tissues and finally anoxia of the tissues.

One of the experiments from among the earliest reported observations on tissue asphyxiation as a feature of shock was as follows:

Experiment with S. C. Harvey, May 21, 1908. The abdomen of a healthy, vigorous dog was opened widely under ether anesthesia; and the viscera were handled for 2 hours in a current of air warmed and moistened with steam. Shock was by that time well developed; and the circulation was approaching failure. Analyses of the arterial and venous bloods—the latter from the right heart—at the beginning and near the end of the experiment showed their contents of oxygen and carbon dioxide in volumes per cent to be as follows:

	ARTERIAL		VENOUS	
	O_2	CO_2	O_2	CO_2
At the beginning.....	15.9	37.4	15.2	39.4
Near the end.....	15.8	16.1	00.0	33.1

Showing complete venous anoxemia, i.e., oxygen starvation of the tissues.

At about the time that these analyses were being made, another series of observations, by Henderson and Barringer, by a different method were also recorded. For those observations the thorax, not the abdomen, was opened; the heart was enclosed in a cardiometer and its volume curve was recorded graphically, while the pressure in the great veins near the heart was measured. The experiments were not intended to induce shock: quite the contrary. Yet most of the animals (vigorous dogs) sank sooner or later into that condition essentially as in the experiments in which the abdomen was opened and the blood analyzed. The succession of events was as follows: (1) A progressive lessening of the venous return and pressure, and a consequent decrease in the rapidity and volume of the diastolic fillings and systolic discharges of the heart, with a more and more thready arterial pulse, but as yet no lowering of arterial pressure. (2) When the venous return and pressure had fallen to such an extent that the strokes of the heart were reduced to a third or less of their initial volume, and the output per minute was correspondingly diminished, arterial pressure fell rapidly. (3) If, at this point, a liberal amount of saline was administered intravenously, the strokes of the heart returned immediately

to full normal amplitude and arterial pressure rose again to the normal level: showing that neither the heart nor the vasomotor system had failed. (4) Soon, however, the saline leaked into the tissues and the succession of decreasing venous return, decreasing stroke volumes and falling arterial pressure recurred. Another infusion again restored both venous flow and arterial pressure; and this could be repeated again and again, until the total volume of fluid that had escaped from the capillaries was so large that the tissues, especially the muscles, were waterlogged.

These two series of experiments, performed 30 odd years ago, showed clearly—for the first time, I think—that a failing circulation leads to a state of venous anoxemia, oxygen starvation of the tissues, leakage of fluid into the tissue, and decreasing blood volume. In recent years this conception has received support from studies on capillary permeability and lymph formation by Landis, Maurer, Drinker and others which show that asphyxiation of the tissues renders them capable of abstracting fluid from the blood almost without limit. As Drinker and Yaffey summarize the evidence, "Just as soon as the circulation rate begins to slacken, the possible effects of oxygen lack on the permeability of the blood capillaries begin to assert themselves." According to Landis the increase of permeability may be as much as four fold.

The sequence indicated by these observations is as follows: When the tonic influence of the motor centers fails, the skeletal musculature becomes flaccid. Because of this flaccidity, the intramuscular pressure falls; the capillary pumps cease to operate; and the venous return to the heart diminishes until it becomes insufficient to allow the heart to maintain the arterial blood stream and pressure. So the first, or circulatory, stage of shock develops.

When in this way the circulation, particularly through the muscles, has decreased to a volume-flow insufficient to supply the minimum oxygen requirements of the tissues, tissue asphyxia develops. Under the influence of this asphyxia a leakage of serum from the capillaries sets in and the blood volume is further progressively decreased. So the second, or asphyxial, stage of shock develops.

Such appears to be the causal sequence through which failure of the tonic activity of the motor centers either because of pain or toxemia in acute diseases, initiates the development of peripheral circulatory failure. That this sequence is essentially correct is confirmed by the benefit which is now obtained, at least in traumatic shock and particularly after severe burns, from the intravenous administration of serum. In such cases, if accompanied by little or no hemorrhage, the red corpuscles, which are the oxygen-carrying portion of the blood, are still in the body, but stagnant, and need only serum to float them. The benefit afforded by mere serum serves to distinguish failure of the circulation of this type from that induced by hemorrhage; for serum alone, even when reinforced by inhalation of oxygen, as it should be, can transport little oxygen. After an exsanguinating hemorrhage, on the contrary, as will be demonstrated in the next section, the essential and only means of saving life is restoration of at least a considerable part of the red corpuscles by infusion of whole blood.

VII. HEMORRHAGE AS A FORM OF ASPHYXIA

Loss of any large volume of blood weakens the circulation. For that fact the elementary principles of hydraulics offer a sufficient explanation. But that is not the chief problem of hemorrhage.

The problem is rather why, after that initial weakening has occurred without causing immediate death, the weakness often increases progressively up to a fatal ending after many hours or even days. There is no surgical service anywhere as yet so perfect as not to have cases of such delayed postoperational deaths. The idea to be here presented is that many of them are essentially post-asphyxial.

In these cases there is a striking similarity to the cases of late death after carbon monoxide asphyxia: deaths occurring many hours or even days after a temporary asphyxia. The blood may then contain no trace of the asphyxiant gas; and inhalation of oxygen is of no benefit. Death is due to a pathological process initiated by asphyxia: a process of which the severity is determined by the length of time that the asphyxia has been allowed to continue. It was the realization of the crucial importance of cutting that time as short as possible that led us (Henderson and Haggard) to develop the treatment of carbon monoxide asphyxia that has now largely eliminated the once numerous cases of postasphyxial death. But, it will be asked, how can the same sort of solution apply to the problem of post-hemorrhagic deaths?

Virtually all of the oxygen that ever reaches the tissues is brought to them in the hemoglobin of the red corpuscles carried in the blood. The amount of oxygen in simple solution in the serum is almost negligible: even under inhalation of oxygen it is small. Blood which has been deprived of any considerable part of its red corpuscles is therefore as lacking in its capacity to fulfill this prime function as is blood in which a corresponding proportion of its hemoglobin is combined with carbon monoxide. The obvious inference from this similarity is the importance of restoration of at least a considerable part of the corpuscles that have been lost, and the equally great importance of effecting this replacement at the earliest possible moment. In other words, the asphyxial period should be cut as short as possible.

In practice the importance of this matter is obscured by the fact that the immediate symptoms of exsanguination appear to be quite as much due to lack of volume of blood as to lack of oxygen. They are largely relieved temporarily by infusion of nearly any fluid. A mere saline solution induces an immediate and strikingly beneficial effect. But the benefit is usually only temporary. Soon the blood vessels are again found empty: the venous return is again insufficient; and arterial pressure again falls dangerously low. The fluid has passed from the vessels, chiefly the capillaries, into the tissues.

If instead of a saline solution one of acacia gum bicarbonate saline is used the beneficial effect lasts very much longer. In fact, in an extensive series of experiments which Haggard and I carried out on dogs several years ago the apparent benefit sometimes lasted for several hours or even into the next day. But within a couple of days all the animals so treated were dead. On the other hand,

both in animals and men, if the lost blood is within a short time replaced by a transfusion of whole blood, at least the animals in our experiments always recovered completely and survived.

Blood contains more than half its volume of serum (plasma). And at the present time, after a considerable loss of blood, as much or even more importance is often imputed to the loss of serum as to the loss of corpuscles. In support of this view is the recent experience, such as that at Pearl Harbor, showing that human serum is of much greater value as an infusant than any artificial fluid yet devised. The reason is supposed to be that the proteins of serum afford an osmotic pressure which prevents the rapid passage of the serum from the blood vessels into the tissues.

Against this conception, there is, however, experimental evidence of a quite conclusive character. It is afforded by a procedure called "plasmapheresis" in which blood is first withdrawn in considerable amounts and centrifugalized. The whole mass of corpuscles is then suspended in a saline solution of exactly the same volume as the separated serum, which is discarded, while the saline suspension of corpuscles is introduced into the circulation. Under these conditions the saline solution does not leave the blood vessels. The whole procedure can be repeated several times. Yet the animal suffers no serious effects. Evidently lack of oxygen is a more potent cause of leakage of serum into the tissues than is loss of the serum proteins.

The necessary inferences from clinical and experimental evidence taken together are that while (1) serum is the most effective immediate infusant, and (2) its value is largely due to its content of albuminous substances, nevertheless (3) the prevention, or at least the abbreviation of hemorrhagic asphyxia, and the avoidance of its sequelae require that at the earliest possible moment the infusion of serum should be followed by a transfusion of whole blood, and that this transfusion be continued until at least 70 per cent of the normal hemoglobin in the patient's blood is reached. And this should not be guessed at, but determined by hemoglobinometer.

Such a weighed consideration of the use of serum and whole blood, and of the quantitative fulfillment of its requirements are diametrically opposed to statements contained in several recent publications, of which the following by Riddell, is an example:

"The use of normal serum for restoring the blood-volume in shock has the same advantages as whole blood in so far as it has the same serum protein content. But in addition, owing to its diminished bulk, it can be given in greater quantity. In other words, the osmotic and therefore the blood-volume restoring properties of a pint of serum can only be obtained by twice that volume of an unseparated blood, a quantity which in all probability it would be unsafe to introduce."

Against such uncritical acceptance of serum as fully equivalent, or even superior, to whole blood for all forms of peripheral circulatory failure, the official publication of the British War Office on "Resuscitation" states: "Blood is the most physiological fluid for restoring blood volume in cases in which the

reduction in circulating fluid is due to blood loss, in that there is the added advantage of oxygen carrying power not possessed by blood derivatives, such as plasma or serum." Serum is accepted as equal, or even superior to whole blood, only after loss of plasma, but not of corpuscles, due to burns. Citrated whole blood—this publication states—can be well kept "for at least 4 weeks."

In the course of our study of hemorrhagic asphyxia several accessory points developed. One was that counting the so-called "rate of breathing" is quite unreliable as a basis for prognosis after hemorrhage. On the contrary measurement of the volume of air breathed in liters per minute is highly significant and reliable. We found that by this criterion the outcome in practically all post-hemorrhagic cases could be foretold. If, after the loss of blood had ceased, the minute-volume of breathing gradually decreased toward a normal resting figure, under any treatment, or none, the animal always survived. If, on the contrary, the minute volume gradually increased, the animal always finally died. In this respect, a slightly, but steadily, increasing or decreasing minute volume of breathing is a better basis for prognosis after hemorrhage than is arterial blood pressure.

In this investigation the amount of blood withdrawn over a certain time was adjusted so that, if the animals were thereafter left to themselves, approximately half of them died, and half survived and recovered. But, if during the hour following the completion of the hemorrhage, even a moderate dose of morphine was administered, all the animals so treated died. If patients who have lost much blood during a surgical operation are similar, it is vitally important that they receive an ample transfusion, not merely of serum, but of whole blood at least as soon as their sufferings are relieved by means of morphine. As the barbiturates are also respiratory depressants, their use during hemorrhagic asphyxia should be avoided. Anoxemic hyperpnea should be counteracted by means of red corpuscles rather than drugs.

Correlated with any increase in the minute volume of respiration after hemorrhage there is always a decrease in the carbon dioxide and bicarbonates of the blood: the two elements always increasing and decreasing in a nearly unvarying constant proportion. A state of low blood alkali is the condition which is now commonly called "acidosis." But, as its physiology—in contrast to its physical chemistry—is still largely unsolved, it is better termed "acarbia," which means simply subnormal carbon dioxide and bicarbonate and is free from any implication as to how the lowering has been induced. It is mentioned here because it is probably concerned in those pathological tissue changes in shock to which Moon has particularly directed attention. Among the disorders in which acarbia occurs are mountain sickness, carbon monoxide asphyxia, asphyxia pallida neonatorum, the dyspnea of extreme muscular exertion, cardiac dyspnea, the process of drowning, diabetes mellitus, the ketoses of young children and, in addition, shock and post-hemorrhagic asphyxia: a group of conditions that should be suggestive for future fundamental investigations.

Large amounts of serum are now being collected by the Red Cross and processed for use in military surgery. The corpuscles are usually discarded. Yet,

if laboratory findings as to the harmlessness of plasmapheresis could be duplicated in the clinic, some at least of those corpuscles, after being thoroughly washed and suspended in a saline solution, could be extremely useful in transporting oxygen from the lungs to the tissues in anemic non-military patients. And this in fact, as I have just now learned, is being done on an extensive scale in Detroit under the direction of Dr. W. B. Cooksey, Technical Supervisor of the American Red Cross Blood Donor Service, in association with Dr. H. L. Alt (q.v.).

VIII. VASOMOTOR AND VENOPRESSOR CONTROLS OF THE CIRCULATION

Our lives are one long succession of adjustments to our environment: adjustment to mental work, to muscular work, to getting and eating food, to escaping the dangers on our streets, to meeting our associates and competitors, to withstanding the cold winds of January and the heat waves of July. All of these and other adjustments, external and internal, are supervised and controlled, partly consciously, but largely unconsciously, by the nervous system. And in very many of them the adjusting reactions take place in the circulation and its coadjutant the respiration.

Heretofore the adjustments in the circulation have been supposed to be controlled only through the nerves to the heart which vary its rate of beating, and through the vasomotor nerves (including under "vasomotor" all of the autonomic system, both sympathetic and parasympathetic), aided by certain hormones, which constrict and relax the blood vessels, both arteries and veins. Now, however, that a third mode of nervous control over the circulation is demonstrated in the venopressor mechanism, it becomes important to differentiate between those adjustments of the circulation which are mainly vasomotor (in this broad sense) and those which are mainly venopressor.

The outstanding differences between them are very simple. Vasomotor reactions are mainly concerned with the adjustment of arterial pressure, and distribution. We should therefore expect mental strain and exertion to induce their characteristic effect of increased arterial pressure mainly through the vasomotor nerves and their chemical necessory, adrenalin. Whether or not thought is a form of energy and consumes oxygen, it certainly requires more blood to the brain. At the same time, however, there is no great increase of total oxygen consumption in the body and therefore no great increase in the minute-volume of the circulation or in the volume of respiration. Little venopressor reaction is needed, or occurs.

Venopressor reactions, on the contrary, are mainly concerned with the adjustment of the volume of the venous return and so with the total minute-volume of the circulation. Muscular strain and exertion may involve a huge increase in the volume of oxygen consumed in the body, and a correspondingly great increase in the volume-flow of blood: that is, an increase in the number of times per minute that, on the average, each red corpuscle makes the round of the circulation and delivers in the tissues the load of oxygen that it gets in the lungs. These are the outstanding differences between venopressor and vaso-

motor adjustments. But, as in the animal body nearly every action anywhere influences nearly all processes and states everywhere, so probably no vasomotor adjustment ever occurs without some venopressor accompaniment, and vice versa.

Two contrasting experiments may illustrate the difference in the adjustments in the circulation under mental strain and those under muscular exertion. At a time when a certain field of physiology had recently undergone important developments, it was my job to try to explain those advances to some 20 second-year medical students. I decided to use my difficult lecture as an experiment on the effect of mental exertion on the circulation. It consisted in having the arterial pressures of both the students and the teacher (myself, then aged 35) measured just before and immediately after the lecture. Also, I noted whether they or I gave any signs of appreciably increased consumption of oxygen, and increase in respiration or in the rates at which the blood went around our circulations. There were virtually none: certainly no one at the end of the lecture was out of breath. But as regards arterial pressure, we all developed a considerable increase. No one of the students failed to show—and I made several of the measurements myself—a rise of at least 20 mm. Hg.: generally more. My own pressure rose from 130 mm. before the lecture to 190 mm. at its end.

In contrast with those vasomotor effects were the observations that Haggard and I once made on the absolute maximum of venopressor adjustments in the members of a university crew: a crew that distanced all competitors in this country and won the Olympic championship abroad. We studied those men throughout the training period, determining the maximal external power of each man by 3 different methods as from 0.45 to 0.57 horsepower and their total internal energy expenditure as 19 to 30 calories per minute, or 13 to 20 times the basal rates. Their oxygen consumption was from 3.5 to 4.0 liters per minute, and the minute volume of air breathed was 80 liters or more: 10 times the resting amounts. Of course, under the exertion of a boat race arterial pressure always rises, but certainly not because of any vasoconstriction; for, if there had not been a considerable vasorelaxation, no vessels could have withstood the enormous volume of blood—probably 25 or even 30 liters a minute—pumped into the arteries by the heart, and the same volume pumped into veins by the booster action of the powerfully working skeletal muscles.

In contrast again to that venopressor adjustment is the reaction that the circulation makes in response to the heat of a Turkish or Finnish bath—hot air or steam. In that adjustment—certainly mainly vasomotor—the skin is flushed with blood; and water from the blood pours through the sweat glands to the extent of liters, or even gallons, per hour.

Contrariwise, again there is syncope. How are we to explain the temporary loss of consciousness, and the flaccidity of the muscles that allows the fainting man or woman to slump to the floor. Is the adjustment, or failure of adjustment, of the circulation in this stage primarily vasomotor or venopressor? Following Lauder Brunton the vasomotor explanation has always been accepted. But a venopressor reaction is more probable. A sudden cessation of the tonus

of all the muscles of the body stops in almost instantaneous succession: (1) the supply of blood to the right heart, (2) the output of the left heart, (3) the pressure of blood in the arteries, and (4) the blood supply to the brain. And failure of the spinal motor centers with loss of muscle tonus may do this quite as effectively as any failure of the vasomotor center and mechanism could. Indeed, there is now virtually conclusive evidence that in life the vasomotor center never fails and would not induce the conditions of either syncope or shock, if it did: at least I should so interpret it. That evidence is afforded by the work of W. B. Cannon and again by B. Cannon. Although not so interpreted by them, it demonstrates that in animals complete surgical removal of the entire sympathetic nervous chain induces no such effect as syncope or shock. Instead, they report that animals upon which this radical operation has been performed "continue to live without apparent difficulty;" that is, with no signs of failure of the venous return. Of course in a man who had lost the central control of the peripheral vasomotor mechanism the ability to withstand a July heat wave would be greatly—perhaps fatally—impaired; and as shown by the literature summarized by Gellhorn in his recent review of "Autonomic Regulations," sympathectomized animals show less resistance to hemorrhage than do normal controls.

In regard to the autonomic regulation of the circulation, it is only fair to the memory of Claude Bernard to point out that he is not responsible for the present general misunderstanding which confuses venopressor phenomena with those upon which Bernard based his classic conception of vasomotor control.

One of the questions that has always puzzled investigators has been: When the circulation fails without a hemorrhage, where has the blood gone? On the assumption that the failure is vasomotor, the explanation offered was that "shock is a hemorrhage into the veins;" or again it was said that "in shock the blood is pooled in the relaxed splanchnic area." But in fact the veins are not over filled—rather the contrary. And unless the abdominal viscera have been exposed, they are not congested.

The venopressor conception of failure of the circulation eliminates this puzzle. The blood need go nowhere to any considerable extent other than where it was before the failure. It simply slows and then stops circulating. It stands for the most part essentially where it was when flowing: the boosters no longer push it onward, the veins collapse, and the asphyxial tissues absorb the serum.

One of the conditions in which fainting is frequent and is certainly due to venopressor failure is altitude sickness. As one sees it among unacclimatized tourists on Pike's Peak (altitude 14,100 feet) the causal sequence is (1) anoxia, (2) overbreathing, (3) acapnia, (4) depression of spinal motor centers, (5) loss of muscle tonus, (6) loss of booster activity and (7) failure of the venous return. Inhalation of oxygen prevents this sequence, and inhalation of even a low percentage of carbon dioxide reverses it. (For similar findings in the laboratory see Gellhorn.)

Essentially the same reversal of anoxic acapnia is an important feature of resuscitation from carbon monoxide asphyxia by inhalation of carbon dioxide

and oxygen. The restoration of muscle tonus and consequent increase in the venous return under the influence of the carbon dioxide is initially more helpful toward the recovery of the patient than is the more slowly developing elimination of carbon monoxide and gradual renewal of the supply of oxygen to the tissues.

In all the principal forms of asphyxia—including drowning, as found by Lougheed, Janes and Hall—the production of carbon dioxide stops instantly that the supply of oxygen in the blood is exhausted. The carbon dioxide in the blood increases therefore only momentarily; and then, strange as it seems, it actually decreases: passing presumably into the tissues. Under this peculiar form of acapnia the venopressor mechanism fails; and inhalation of carbon dioxide is needed to restore the circulation as well as respiration. Asphyxia does not consist of anoxia and hypercapnia as was long believed, and is now again claimed by Gellhorn and by Van Liere. Asphyxia of that sort can be produced experimentally; but no such state occurs outside the laboratory. All clinical asphyxias consist of anoxia and the resulting acapnia, either from over-breathing or by passage of carbon dioxide from the blood into the tissues. Gellhorn's interesting tests of anoxia by means of handwriting show that in asphyxia acapnia is more immediately disturbing than is anoxia alone without acapnia.

Finally, among the vasomotor and venopressor reactions and adjustments or maladjustments of the circulation, by far the most important are those which occur in such cases as that of old Sir John Falstaff quoted in Section II. The primary failure is in the spinal motor centers. It occurs gradually as the dying patient grows weaker. The muscles lose their tonus, and intratissue pressure. The booster pumps in the bundles of muscle fibers beat more and more feebly. Less and less blood is supplied to the right heart and correspondingly less is available to be pumped into the arteries by the left heart. As the arterial stream diminishes the vasomotor mechanism makes a compensatory effort to maintain sufficient arterial pressure to supply blood to the brain. And when further compensation is no longer possible, the final and fatal fall of arterial pressure and stand-still of the circulation occur.

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SERUM SICKNESS AND ANALOGOUS REACTIONS FROM CERTAIN DRUGS PARTICULARLY THE SULFONAMIDES

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At the present time it seems desirable to review the entire subject of serum disease. The problem requires, rather urgently, reconsideration because of the efforts at the present time to find substitutes for human plasma transfusions; and for the reason that the toxic reactions following the therapeutic use of a number of drugs, particularly the sulfonamide group, resemble in certain respects serum sickness.

It must be recognized that our knowledge of serum disease has been acquired almost exclusively from observations upon man, and starts essentially with the classical work of von Pirquet and Schick (1) in 1905. Though the condition had been recognized long before, it is justifiable to date the modern conception of the disease from their important monograph. Since then the publications upon serum disease and its related phenomena have been legion; and as the essential data have been collected in innumerable statistical articles, reviews, monographs, and textbooks, it is entirely unnecessary to reiterate them at this time (2, a, b, c, d, e, f, g, h, i, j, k, l, m).

It is desirable, however, to point out that the statistical compilations are unavoidably based on results obtained from the use of whole blood serum, or concentrated antisera of many varieties, employed for the treatment of different sorts of people, suffering from a number of diseases. It is at once apparent that the many variables impair the usefulness of these statistics for accurate analysis.

It is particularly important to keep this in mind in dealing with some of the components which go to produce serum disease, such as the antigen or sensitizing substance. The antigens employed so far, as compared with those studied in experimental anaphylaxis, make rather a short list. They consist largely of the proteins which are present in serum from horses immunized to a variety of toxins and bacteria, or of these antisera, concentrated and refined; so that the greatest amount of antibody or antitoxin is contained in the smallest amount of serum. The sera of other artificially immunized species, such as the rabbit, the beef, the sheep, the goat and the chicken have also been employed; but the vast majority of observations have been made after the use of horse serum, either for prophylactic or therapeutic purposes.

Blood serum contains at least four serologically distinct species specific proteins; namely globulin, fibrinogen, serum mucoid and albumin (2 k). The exact physical methods of analysis by ultracentrifugation and electrophoresis in the Tiselius apparatus have shown, however, that the composition of normal animal serum and human serum consists of albumin and three forms of globulin —known as alpha, beta, and gamma globulin. The albumin predominates while the gamma globulin is present in next largest amounts. Though the

other protein fractions are present in much smaller amounts, this quantitative difference may not be commensurate with their importance.

The relative proportions of these protein constituents are found to be different from the normal in the serum from immune animals. Analytical ultracentrifugation of antipneumococcus horse serum has shown that the usual pneumococcal antibodies differ in weight from the components in sera from normal horses (3). The electrophoretic studies by Tiselius and Kabat (4) of antipneumococcus sera from various animals led them to conclude that the antibody in immune rabbit serum migrated with the gamma component of normal serum, whereas the antibody in immune horse serum migrated as a new serum component between the beta and gamma components. Further analyses by the electrophoretic method of a great variety of antibacterial and antitoxic sera (5) have disclosed the fact that in some the antibody was indicated by an increase in the amount of gamma globulin normally present, while in others the antibody was expressed by the appearance of the new T. component.

The different proteins of normal serum are separable, not only by their chemical constitution and physical properties, but by their ability to act as specific antigens; for Dale and Hartley (6) have demonstrated that anaphylaxis discriminated between three purified proteins separable from normal horse serum by chemical methods. A "guinea pig which received a preparatory injection of the pure euglobulin became sensitive to this, but remained indifferent to the pure albumin and vice versa." There was a difference also in the time of appearance of sensitivity to the proteins employed; for sensitiveness to euglobulin appeared within 8 to 10 days after injection, while sensitiveness to albumin was not demonstrable until after an interval of 16 to 20 days. Doerr and Berger (7) have found analogous differences between the protein fractions of horse serum, while Taylor and Keys (8) have confirmed these observations for the albumin and globulin fractions of bovine serum.

It seems obvious from these analyses of blood serum that when the whole serum from normal animals is injected into human beings the fluid contains several specific antigens. Furthermore when the serum from immune animals is employed the antigenic property of these sera may be distorted from the normal by the predominance of one protein fraction over another, or possibly by the actual appearance of fractions not readily demonstrable or absent in normal sera.

These facts have long been recognized, for Hooker (9) found that the skin of patients, who had been sensitized to horse serum by a small prophylactic dose of diphtheria antitoxin, reacted differently to the intracutaneous injection of three protein fractions of horse serum; inasmuch as the wheal produced by pseudoglobulin appeared in 30 minutes, that caused by euglobulin in 5 hours and that elicited by albumin not until 7 hours had elapsed. It appears, moreover, from the observations of Seegal (10) and her associates that individuals, supposedly sensitive to horse serum, may react quite differently when small amounts of the same form of antisera, derived from different animal species, are injected intradermally. They report that Type I antipneumococcus horse

serum produced protracted severe local reactions in 8 of 12 such individuals, whereas equine tetanus antitoxin globulin elicited only slight reactions. Type I antipneumococcus rabbit serum caused a slight reaction in only one individual, while antistreptococcus haemolyticus sheep serum brought forth a severe reaction in 9 of the individuals.

Although the differences may be due in part to the changes brought about in the serum through active immunization of the animals or be the result of the refinement of the sera, still, there is some evidence to show that the sera from some species are not as likely to cause serum sickness as the sera from other species. The statement of Kraus (11) that bovine serum is less likely to be antigenic for human beings than horse serum has often been quoted. It has also been said that fowl serum, freed from fibrinogen, does not produce serum sickness when injected into human beings (12).

It is for reasons such as these that studies have recently been made upon bovine plasma and serum in hopes that either one or the other might be used to replace transfusions of human plasma. Wangensteen (13) and his associates administered 750 cc. to 1000 cc. of bovine plasma over a period of 4 days to 4 normal individuals. Three of these individuals developed urticaria 5 to 6 days after the injection, which persisted for 3 to 4 days. Davis (14) and his associates transfused bovine serum albumin in amounts of 50 to 300 cc. to 13 human beings. There were no immediate reactions, but there is no statement as to whether or not serum disease resulted later. Taylor and Keys (8) report immediate urticarial reactions and collapse after both the first and second intravenous injection of 6 to 8.8% bovine albumin solution. On examination of these preparations in the Tiselius apparatus they found that the electrophoretic pattern of the solutions showed a component moving more slowly than albumin. They suggest that this fraction may have contained the antigenic substance. The results obtained by Janeway and Beeson (15), however, show that most carefully purified bovine albumin is antigenic, and will produce serum disease in man; for amongst 16 normal persons injected intravenously with various amounts of this purified material in solution, two developed typical serum sickness. Although the hope still exists that repeated purification of bovine serum albumin may furnish a fluid rich in protein, and be, at the same time, innocuous for human beings, this has not yet been accomplished.

As a converse of these experiments with bovine serum, attention must be called to the fact that under certain circumstances the antigenic property of antitoxic and antibacterial sera may be enhanced by certain procedures. Hooker (16) observed that children were readily sensitized to minute quantities of horse serum, when this was combined with diphtheria toxin in toxin-antitoxin mixtures; for it was found that 64% of children injected with the toxin-antitoxin mixture showed sometime later positive skin reactions to 0.02 cc. of horse serum. Park (17 a) has confirmed these observations and reports that 37.7% of 116 children receiving three injections of toxin-antitoxin mixture, which contained only 0.0002 cc. of globulin, gave positive skin reactions subsequently. Some of these patients also reacted violently to later intravenous injections of

antitoxin so that the sensitization induced in this manner may be general and of extremely high degree. We have observed, while performing neutralization tests, that violent local reactions in the skin of normal individuals may be brought about by repeated intracutaneous injections of mixtures of filtrates from haemolytic streptococci with minute quantities of antistreptococcus horse serum. The amount of horse serum actually injected in each test was 0.0001 cc. Freund and McDermott (17 b) have noted that the use of adjuvants such as "aguaphor" modified the sensitization of guinea pigs to horse serum in several ways. Reactions following intracutaneous injection were necrotic and lasted more than 48 hours; the animals remained highly sensitive for an unusually long time, and precipitins appeared in the blood of the guinea pigs in much higher titer than in control animals, sensitized to serum alone.

The problem of serum disease is still further complicated by the fact that reactions precisely like those of serum sickness have been occasionally observed in man after transfusion of homologous blood or plasma, or following the injection of homologous serum. Dooley's (18) patient subsequently showed positive skin reactions to the particular human serum employed and specific antibodies for this human serum were demonstrable by the Prausnitz-Küstner tests. Fox (19) records 1% reactions, the exact nature of which is not clear, in 420 cases of scarlet fever receiving human pooled convalescent serum. These reactions, occurring after the injection of human convalescent sera, have usually consisted of fever, urticaria, arthralgia, lymph adenopathy, or nausea and vomiting and have often appeared within 3 to 5 days after injection. Goebel (quoted from Voss) has observed local reactions which have appeared 12 days after injection of normal human serum, employed for the prophylaxis of measles. Occasionally reactions simulating serum disease are seen after blood transfusions. The cause of these reactions following the introduction of homologous serum or blood into human beings is very obscure.

Indeed the specific nature of antigenic substances has for many years attracted the attention of investigators. The demonstration finally that the specificity of antigens depended upon their chemical constitution opened the way for some of the most important advances that have been made in the whole field of immunology (20, a, b, c, d, e). The entire subject has been extensively reviewed by Landsteiner (2 k) who is one of the great pioneers in this field. It would be impossible in this paper to discuss such a highly complicated problem, but suffice it to say that antigens, proven to be immunologically active, can be artificially constructed by conjugating pure chemicals with animal sera or with other proteins. This combination results in an antigen possessing a high degree of specificity. The specificity of these antigens has been shown through innumerable experiments to depend upon the chemical radical and not upon the protein fraction of the conjugated antigen. For this reason an animal may be sensitized to an antigen formed from conjugation of its own blood serum with one of many chemicals. The animal then becomes sensitive to this new conjugate, or, in many instances, to the chemical or radical itself. Landsteiner (23) in his investigations with Jacobs and Chase has recently shown that guinea pigs

may be sensitized with a pure unconjugated chemical. This can be accomplished by application of the chemical to the skin, or through a special technique, by intraperitoneal inoculation; so that, after an interval of 3 weeks, application of the pure chemical to the skin produces a typical reaction while the intravenous injection of the chemical conjugated with protein causes anaphylactic shock.

Hypersensitivity to a long list of drugs and chemicals has for many years been familiar as a common form of allergy in human beings. It is now known to be one of the most frequent industrial hazards, for workers in many factories are prone to develop "contact dermatitis" from handling several varieties of dyes and chemicals. It is, therefore, important to note that the pure chemicals with which Landsteiner experimented, among which were picric acid; 2:4 dinitrochorobenzene, uroshiol and related compounds (contained in Japanese lacquer), and 1:2:4 chlorodinitrobenzene, are substances which have been described as common incitants of contact dermatitis in man. The experiments with mustard oil (24) were particularly interesting; for though it was not possible to sensitize guinea pigs, rabbits or monkeys to this chemical, it was possible to obtain cutaneous sensitivity in one man and in hogs by repeated application of the chemical to the skin. It is especially important for our problem that Landsteiner and Jacobs (25) were able to sensitize guinea pigs to arsphenamine and neoarsphenamine so that subsequently typical skin reactions could be obtained by intracutaneous injection, and anaphylactic shock was produced by intravenous injection of the pure chemical uncombined with protein.

These experiments offer an adequate explanation for the sensitization of the human being to pure chemicals. It is supposed that repeated contact with a given chemical such as formalin (21, 22) leads eventually to the formation of an antigen constructed from a combination of the chemical with the body proteins of the individual. After this has taken place, an allergic reaction results from intimate contact with the chemical. If the reaction is an immediate one, it is probably caused by the chemical alone; if the reaction, on the other hand, is slow in onset it is more likely that the conjugation of the chemical with the body protein is necessary to bring about the reaction.

There are three forms of chemicals among others that produce an intoxication, the symptoms of which appear roughly within a week to 10 days after the start of the first course of the drug. In this respect, and in many others to be discussed later, the intoxication resembles serum sickness. The drugs are Nirvanol, the arsphenamines and the sulfonamides.

It has already been pointed out that guinea pigs may be sensitized to arsphenamine and neoarsphenamine. It now appears from the work of Wedum (26) that guinea pigs can also be sensitized to azoproteins prepared from the sulfonamides. The reactions are not, however, absolutely specific, for there may be interreactions between the different sulfonamide compounds. Davis (27 a) has shown that the proteins of human plasma, more particularly serum albumin, will bind the sulfonamides leaving only a portion of the drug free to dialize. There is some indication also that the drug bound to the protein is no

longer bacteriostatic (27 b). The combination takes place much more readily with sulfathiazole than with the other forms. This is interesting in view of the fact that this drug results very frequently in intoxication.

The Mechanism of Serum Sickness. It has already been pointed out that the variables incident to the employment of the antigen are very great, and it may be said that they grow equally numerous in the process of sensitization. Among these the age and race of the patient; his previous contact with the antigen employed; his individual susceptibility, and the method of administration of the serum are important. In general children (28) are more likely to have serum sickness than the aged, and negroes are thought to be less susceptible than the white race; but this may depend in part upon the fact that skin eruptions are not as easily detected in the black as in the white skin. Coca (29) found American Indians less likely to develop serum sickness than the white race. It has been pointed out by Tuft and Ramsdell (30), however, that Coca employed normal horse serum in his experiments, and that the reaction of human beings is somewhat different to normal horse serum than to antitoxic and antibacterial sera. They found that the formation of antibodies to horse serum, which occurs regularly when antisera are used, is negligible or absent when normal sera is employed.

The amount of antigen injected plays some part, but there are individuals in whom serum sickness appears after the administration of small amounts of antigen, and others in whom the intravenous injection of enormous amounts of whole horse serum is not attended by the slightest evidence of serum disease. Subsequent skin tests may, nevertheless, demonstrate sensitization to horse serum. The original statistics of Weaver (31 a), based largely on the use of whole serum, afford as good an index as any other of the incidence of serum disease following the use of various amounts of whole serum. Roughly this mounts with increasing quantities of serum, so that 10 cc. of serum results in an incidence of approximately 10%, while 100 cc. results in an incidence of approximately 90%. Gerlough (31 b) found that the incidence of serum disease following the use of antidiphtheritic serum, computed from the figures published by Weaver and by Hunt (31 c), was closely correlated with the square root of the amount of serum injected. These figures, however, do not give any idea of the proportion of patients that are actually sensitized to the foreign serum, and who, after a lapse of weeks or months, show positive skin reactions, conjunctival reactions or immediate or accelerated reactions, following a second dose of serum. Mackenzie (32) found that fourteen of sixteen patients receiving large amounts of antipneumococcus horse serum intravenously (100 to 1000 cc.) gave positive skin reactions to horse serum when tested two to eight years later. The statistics of Hooker (16) and Park (17 a) have already been quoted, showing that under certain conditions infinitesimal amounts of foreign serum, when injected intravenously or subcutaneously, may sensitize the skin; or, in some instances, produce general sensitization lasting for years.

Though anyone of these many variables may have its effect upon the incidence or course of serum disease, or may make the interpretation of the results of clinical observations and experiments difficult, not one in any way controverts

the present concept of the fundamental basis of serum disease and its related phenomena. Von Pirquet and Schick (1) propounded the idea that serum disease was caused by an antigen-antibody reaction. Though Coca (2 h) once regarded the problem from another point of view, the accumulation of a vast amount of evidence supported by Doerr (2 f), Dale (2 c), Zinsser (2 l) and others upholds the theory that the disease is caused by an antigen-antibody reaction within or upon the cells; so that serum sickness really represents a visible evidence, so to speak, of active sensitization of the individual to n foreign protein. Thus the phenomenon is included in the broad conception of anaphylactic reactions.

Following the injection of large amounts of serum, the tissues react with the formation of a great variety of antibodies. It was originally demonstrated by Hamburger and Moro (33) that precipitins for horse serum appeared in the patient's blood during or following an attack of serum disease. These observations have been repeatedly confirmed. An analysis of precipitin reactions by Longcope and Rackemann (34) showed that when serum disease followed the injection of large or small amounts of antipneumococcus horse serum, precipitins appeared in the serum of the patient sometime during the later phase of serum disease. Towards the end of serum sickness there was usually a sharp increase in the precipitin titer for horse serum, and at this time the antigen, which had been demonstrable throughout the course of the disease, diminished rapidly or disappeared entirely. When serum disease did not occur the precipitins were not detectable in the serum and the antigen or horse serum persisted for long periods of time and could be demonstrated for weeks or even months (Chart I). Mackenzie and Leake (35) and Tuft and Griffith (36) have confirmed these observations. De Gara and Bullowa (37) have quite recently obtained similar results after the injection of antipneumococcus rabbit serum. Mackenzie (38) has found that when serum disease assumes the immediate or accelerated form, the production of precipitins is much more rapid, and the disappearance of antigen is accelerated, taking place at the time that the precipitins in the serum reach a high titer. Furthermore, it is possible to sensitize guinea pigs passively with the serum from patients with serum sickness (30, 34, 36), and to obtain with these sera positive Prausnitz-Küstner reactions (30, 40). It has repeatedly been shown that the skin of these patients, whether or not they have serum disease, becomes sensitized to a second injection of the specific serum, and this sensitivity can be detected by intracutaneous tests shortly after the subsidence of the generalized eruptions.

There are a few accidental observations which show that passive anaphylaxis to horse serum, such as that obtained in the guinea pig, can also be reproduced in the human being. Kojis (2 m) reports an instance of passive sensitization that arose through the use of serum obtained from a patient treated three weeks previously with antiscarlatinal horse serum. Diphtheria antitoxin was injected into the buttocks eleven days after the patient had been treated with the convalescent human serum. A local reaction with high fever began three hours later and progressed to a violent inflammation of the Arthus type.

Under another set of circumstances, what has been termed reversed or in-

verse anaphylaxis, may be produced. In these experiments antigen is injected first and after an interval of several hours or longer, the antibody introduced intravenously. Under these circumstances an immediate acute reaction occurs at the site of the primary injection of antigen; or if sufficient time has elapsed, a generalized reaction takes place. Opie (41) first called attention to this form of anaphylactic reaction in his experiments upon the Arthus phenomenon in rabbits. Reversed anaphylaxis has also been studied by Kellett (42). Reversed anaphylaxis has attracted some attention in human beings. Mills and Schiff (43) reported an instance of what may be considered local reversed

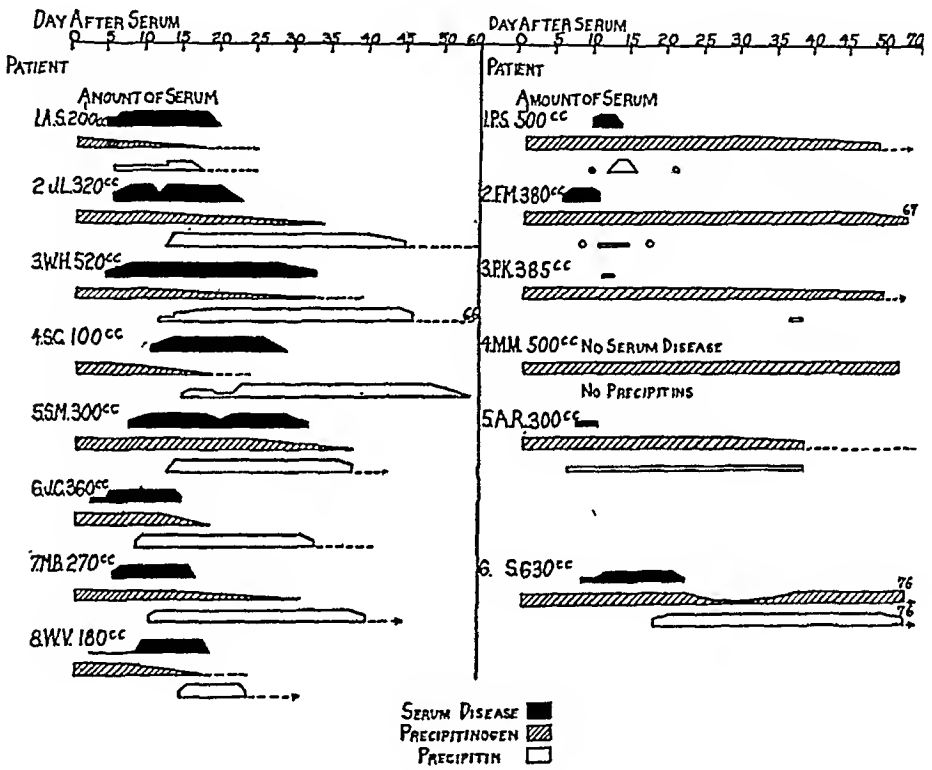

Chart I. Showing the relative proportions of antigen (horse serum) and precipitin for horse serum in the serum of a series of cases of serum disease. It will be noted that when serum disease is mild and of short duration, the precipitin reaction is of low titer and is only obtained for a few days. Antigen, however persists in the patient's serum for long periods.

anaphylaxis in a hemophiliac. The patient, who was bleeding, was found negative to an intradermal injection of 0.2 cc. of normal horse serum; he was therefore given 4 cc. of normal horse serum subcutaneously. The following week he was transfused with 400 cc. of blood from a brother. Eight days after the first intradermal test he was again found to be negative to horse serum and was transfused with 800 cc. of citrated blood from his brother-in-law who was found later to be sensitive to horse serum. Thirty minutes after the completion of the transfusion there was what is described as a "toxic protein reaction" at the site of the intradermal injection of horse serum which he had received six hours previously. This soon subsided but recurred off and on for six days. A more

impressive instance of reversed anaphylaxis was reported by Kellett (44). A child with scarlet fever was given antiscarlatinal horse serum into the right thigh. About an hour afterwards the patient received a transfusion of blood from an individual who previously had had injections of horse serum and was known to have become highly sensitive to horse serum. Within a few minutes after the transfusion an urticarial eruption appeared at the site of the injection of antiscarlatinal horse serum, and somewhat later the child developed a widespread urticarial eruption. It had been demonstrated before the scarlatinal antitoxin was administered that the child's skin was not sensitive to horse serum. Voss (45) has made an extensive study of reversed anaphylaxis, or, as he terms it, "inverse" anaphylaxis in man. His experiments were made with normal horse serum as an antigen. The antibody was contained in the serum of patients convalescent from serum disease, which followed the administration of equine diphtheria antitoxin. The normal horse serum was injected subcutaneously in 1–100,000 dilutions in normal individuals. After a period varying from two to eight hours, 1 to 10 cc. of the convalescent serum was given intravenously. A local urticarial eruption appeared under these circumstances at the site of the previous injection of normal horse serum. If, on the other hand, larger amounts of normal horse serum were employed for the primary injection, and the convalescent serum was administered three to four days later, not only a local reaction occurred but a general reaction resulted which produced in every detail serum disease. Karelitz and Stempien (46) have recently repeated and confirmed these experiments. They were able to precipitate attacks of serum sickness within three days after the primary administration of horse serum in 65% of 62 patients by the intravenous injection of 2 to 10 cc. of serum from patients convalescent from serum disease.

An additional interesting observation was made by Voss (45). He discovered that the local reaction does not depend specifically upon the subcutaneous introduction of horse serum; for he obtained the same result when beef serum and sheep serum were used as antigens. This result is in accord with the statement of Mackenzie (32), that patients sensitized to horse serum may later give heterologous skin reactions to sheep serum, rabbit serum and chicken serum.

Masugi called the form of nephritis which he produced in rabbits, by injection of nephrotoxic sera, a form of reversed anaphylaxis. Kay (47) has shown that the Masugi form of nephritis, caused in rabbits by the intravenous injection of rabbits with serum from ducks, treated over a long period with injections of washed rabbits' kidneys, is closely associated with the production of antibodies to the duck serum itself; for the onset of nephritis in the rabbit occurs at about the same time that precipitins for duck serum are demonstrable in the blood of the test rabbits. If the formation of precipitins is suppressed by the previous treatment of the rabbits with X-rays, duck serum remains for long periods of time in the blood of the rabbit; precipitins do not appear and nephritis does not occur. When, however, during this period, the rabbit is given serum from rabbits that have been injected with duck serum containing a high titer of precipitin for duck serum, an attack of acute nephritis is precipitated in a large proportion of these X-rayed rabbits.

From all the evidence which is at hand at the present time it appears that the fundamental reactions of the tissues of the human being towards the artificial introduction of soluble foreign proteins is of the same nature as anaphylaxis in animals. The ease with which these reactions can be brought about, the susceptibility of different tissues and therefore the manifestations of these reactions vary considerably in different species; but the fundamental principles upon which they depend seem to be practically identical. The Arthus phenomenon, acute and delayed anaphylactic shock, passive sensitization and reversed anaphylaxis can all occur or be induced in the human being. Antibodies appear in the circulation, during or after serum sickness, for precipitins; and anaphylactic antibodies are demonstrable, while the Prausnitz-Küstner reaction can frequently be obtained. The skin and conjunctiva become sensitive and acute anaphylactic shock can occur on reinjection of the specific protein.

Typical serum sickness is, however, peculiar to the human being. It is said by some to occur in calves; and a local reaction in the ear of the rabbit, analogous to serum disease, has been shown by Fleisher and Jones (48) to appear with some regularity in the rabbit following a single injection of large amounts of horse serum.

There is less known concerning the immunological reactions in man to chemicals and drugs, or to the antigens formed by their conjugation with proteins. Landsteiner (2 k) has suggested that certain simple chemicals, after application to the skin or after injection intradermally, combine with body proteins to form true antigens, similar to azoproteins prepared in vitro. Reference has already been made to the experiments of Landsteiner (21) and of Horsfall (22) with formalin. Landsteiner's experiments furnish evidence that chemicals may conjugate with proteins within the body and thus give rise to antigens foreign to the host, and, therefore, capable of sensitizing the tissues of the host. The reaction of the tissues is such that under these circumstances humoral antibodies may be produced in the animal. In man humoral antibodies have rarely if ever been demonstrable following reactions to drugs and chemicals. Specific skin reactions may occasionally be obtained by the "patch" technique.

PATHOLOGY

Until recently there was very little known about the pathological anatomy of serum sickness; but what little information we have gained from the study of anaphylaxis in animals, leads to the conclusion that the process between antigen and antibody results in an inflammatory process which takes place either at the point of local inoculation or, when the injection of antigen is made intravenously, in many organs of the body. The subject has been reviewed by Opie (49) and Heinlein (50).

The characteristic local reaction is the Arthus phenomenon which occurs in man (51) as well as in the rabbit. The histological changes that appear in the subcutaneous tissues consist of an acute inflammation involving the walls of small blood vessels, thrombosis and necrosis of tissue (52). Similar local inflammatory reactions have been obtained in a number of situations. Thus intra-

tracheal injection of the antigen in sensitized rabbits results in a form of pneumonia (53, 54); repeated intraperitoneal injections bring about an inflammatory reaction in the omentum (55, 56), while primary inoculation into the anterior chamber of the eye (57), and joints (58, 59), or the pericardium (60), followed later by an intravenous injection of the antigen, produces an inflammatory reaction in the region of primary inoculation.

Inflammatory reactions in many organs of the body have been observed when foreign sera or egg white have been injected repeatedly intravenously in rabbits, or when these animals have been sensitized to the antigen and subjected later to several anaphylactic shocks (59, 60, 61, 62, 63, 64, 65). The changes have been particularly marked in the kidney, where a form of nephritis affecting the glomeruli has been found in the myocardium and about the blood vessels of various organs. Heinlein (50), in his comprehensive review, emphasizes particularly the vascular lesions. The periarterial collections of cells, the acute necrosis and inflammation of the vessel wall with hyaline degeneration of the media resemble closely the familiar histological changes in periarteritis nodosa. Rich and Gregory (66) have recently reported widespread vascular lesions in rabbits which have received a single large injection of horse serum. The animals were killed during the time at which the reaction in the ears, described by Fleisher and Jones (48) as serum sickness, was at its height. The changes in the blood vessels were considered by Rich and Gregory to reproduce almost precisely those found in periarteritis nodosa in human beings.

All of these observations and experiments lead necessarily to the conclusion that the reaction between antigen and antibody, following the injection of foreign blood serum or other proteins into animals sensitized to the protein, results in widespread lesions of an inflammatory nature. The walls of blood vessels seem to be particularly vulnerable; for widespread acute arteritis is a common finding. These changes can be brought about either by repeated intravenous injections of the antigen or by single large injections into the veins.

The Arthus phenomenon in the human being is obviously an inflammatory reaction. One must suppose, too, that there would be some anatomical change in many tissues of the body during serum sickness; for though the eruptions are ephemeral, the enlargement of lymph nodes is often marked and quite persistent. This furnishes some evidence that the lymphoid tissues are structurally affected. The arthralgia is sometimes accompanied by actual swelling of the joints. Boots and Swift (67), who have studied the fluid aspirated from the knee joints in cases of serum sickness, following the administration of large amounts of antipneumococcus horse serum, find that the exudate is of an inflammatory character. The joint fluid was shown by precipitin tests to contain horse serum.

Death from serum disease or even during serum sickness is so exceptionally rare that there has been little opportunity to obtain information concerning the presence of any possible pathological lesions in the internal organs. It is true that Fleisher and Jones (48) have observed an erythematous and edematous reaction in the ear of rabbits, appearing from 3 to 8 days after the intravenous

injection of a single large dose (5 to 10 cc.) of normal horse serum, but Khorazo (68) was unable to reproduce a similar reaction in rabbits with other foreign sera, such as normal human, sheep, guinea pig, and dog serum. Fleisher and Jones give no description of any anatomical changes in the organs of the rabbits in their experiments. The studies of Rich and Gregory (66) show, however, that this so called serum disease of rabbits is accompanied by widespread periarteritis nodosa.

Two important papers have recently been published upon the histological changes occurring in the organs of man during serum sickness. Clarke and Kaplan (69) had an opportunity to study the tissues from two patients who died during serum disease following treatment with large doses of antipneumococcus horse serum. The distinctive structural alterations consisted in a proliferation of histiocytes in the endocardium and in the intima of the aorta and pulmonary arteries. Necrotizing arteritis and periarteritis of the smaller coronary arteries formed a prominent finding in one case. They considered that the composite pathological picture was not compatible with any disease hitherto described, and concluded that it represented an expression of hyperergy and was related to the administration of the foreign serum.

More recently Rich (70) has been able to add important information to this subject. He has studied the tissues of seven patients, six of whom have come to autopsy. Five of these patients, four of whom died, had received antipneumococcus horse or rabbit serum in combination with sulfadiazine alone or together with sulfapyridine or sulfathiazole. One patient had received antimeningococcus horse serum alone; and one patient had received sulfathiazole alone. The interesting and consistent lesion which he found in all of these patients was an acute arteritis and periarteritis, affecting the smaller vessels of a large variety of organs in the different cases and presenting the pathological picture of periarteritis nodosa. The lesions were similar to those which he and Gregory found in their experimental animals. After a discussion of the entire question, he concludes that vascular lesions of this type can be a manifestation of the anaphylactic type of hypersensitivity in man.

Since five of the patients had been treated with both horse serum and sulfonamide drugs, it seems pertinent to inquire as to what part serum sickness, and what part intoxication from the sulfonamide drugs had in the production of these lesions; or how necessary it might be to have a combination of the two in order to bring about the resulting lesions in the vessels. The question seems to be answered by the fact that one additional patient had received antimeningococcus horse serum without sulfonamides, and another patient had received sulfathiazole without any form of serum therapy. It seems, therefore, justifiable to conclude that either serum disease or intoxication from the sulfonamide drugs may give rise to these acute arterial lesions, which seem to be an expression of an allergic reaction to foreign serum in the one instance and to the sulfonamide drugs in the other.

These publications of Clarke and Kaplan and of Rich leave no doubt but that both serum sickness and intoxication from some of the sulfonamide drugs may

result in a form of periarteritis nodosa; and that the lesions in the two types of disease are the same. This is a very important matter in relation to our understanding of the pathogenesis of the untoward reactions following the administration of sulfonamides and possibly of other chemical compounds.

Since both foreign serum and sulfonamides may cause the pathological picture of periarteritis nodosa, it seemed desirable to ascertain whether serum disease was more common when both factors were operative than when either one or the other therapeutic procedure was employed alone. For this reason we have reviewed, with the aid of Dr. Giles F. Filley, the clinical course of twenty-eight patients who received concentrated antipneumococcus rabbit serum alone, and twenty-nine patients who received either antipneumococcus rabbit or horse serum in combination with one or another of the sulfonamide drugs, usually sulfadiazine. Patients were not included in these series unless they had been observed in the hospital for at least twelve days after the first dose of serum. It was found that the incidence of serum disease in the two groups was very nearly the same, occurring in fifteen of the twenty-eight cases in the first group, or in 53.5%; and in thirteen cases of the second group, or in 44.8%. It seems justifiable to conclude from a survey of this limited number of patients that the addition of sulfonamide drugs to serotherapy did not increase the incidence of serum disease over and above that which might be expected from the administration of antisera alone.

CLINICAL SERUM DISEASE AND DRUG REACTIONS

Serum sickness, particularly in its milder forms, is so common following the widespread use of prophylactic and curative antisera, that it is sometimes regarded as an insignificant accompaniment of these valuable methods of treatment. It may, however, assume quite serious proportions, and it usually results in a state of hypersensitivity to the particular animal serum employed.

As von Pirquet and Schick (1) first pointed out, serum disease appears in three forms. They termed these the "normal reaction," the "accelerated reaction," and the "immediate reaction."

The normal reaction follows the administration of foreign serum to an individual who has not previously had an injection of serum, derived, at least, from the same species of animal; or who is not spontaneously sensitive to this particular protein. Under these circumstances there is an incubation period averaging from six to ten days, though in some instances this may be shortened to three days or lengthened to twenty-one days. In 1,493 collected cases (2 g) the incubation period was eight to nine days in over one-third, and from six to ten days in 1,053. Kojis (2 m) found that in 90% of 1,264 cases the incubation period was less than eleven days, and in 421 of the cases it was six or seven days.

There are rarely prodromal symptoms for the onset is usually abrupt. Malaise, muscle pains, nausea, vomiting and arthralgia are the most frequent symptoms; while the commonest signs are eruptions, fever, edema, lymphadenopathy and arthralgia. These are usually inconsequential except for the

urticaria which may be extremely exasperating and the arthralgia which is always exquisitely painful.

The rashes may be very varied. Urticaria is the first one to appear and is the commonest. It often initiates the reaction by appearing at the point of injection of the original dose of serum, but rapidly spreads to a generalized eruption. The generalized urticaria may be persistent for a few days or may come and go (Chart II). This is sometimes accompanied, or more often followed, by an erythematous or scarlatinal eruption. Morbilliform rashes or

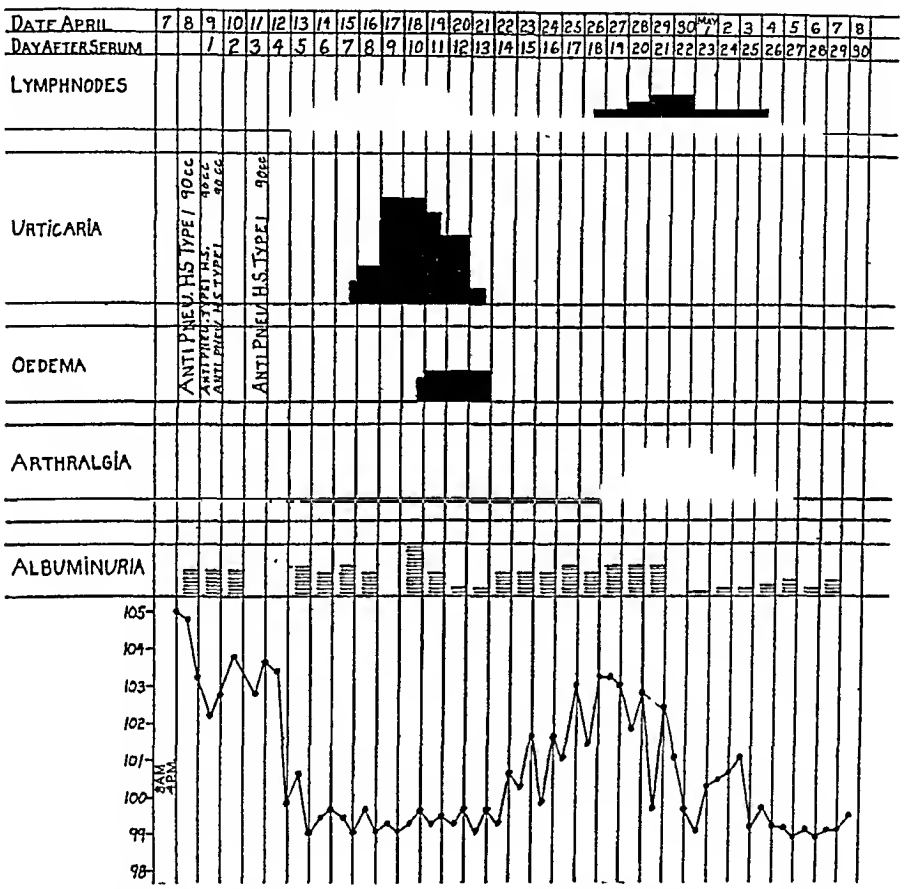

CHART II. Illustrates the clinical course of serum disease following the intravenous injection of large amounts of antipneumococcus horse serum

those of erythema multiforme type are much less common, while crops of petechiae or more extensive purpura are very rare. These hemorrhagic phenomena are not due to thrombocytopenia. Any or all of these rashes may recur once or even oftener. When they do so an interval of two to several days usually intervenes, and the second or third forms of eruption are generally different from the first. Davidson (71) has made an exhaustive study of the various forms of eruptions; and, following the suggestion made by Dale and Hartley, Hooker and others, believes them to be an expression of the reaction to the different forms of protein which go to make up the antisera employed.

Single recurrences are observed in about 3% of cases, double recurrences in less than 0.5% (2 m).

The arthralgia is rarely accompanied by swelling, reddening, or increased heat about the joints. Occasionally an effusion of fluid can be demonstrated in the knee joints. The aspirated exudate is found to contain an increased number of leukocytes indicating an inflammatory reaction, while precipitin tests demonstrate the presence of the specific serum with which the patient was treated (67).

The lymph adenopathy may be localized or generalized. The lymph nodes nearest the point of injection are often the first to enlarge. The swollen lymph nodes are sometimes painful and tender. Occasionally the spleen is palpable.

Edema is common in children. It is particularly noticeable in the face and about the eyes. The edema may not be confined to the subcutaneous tissues, but may affect the lips, tongue, buccal mucosa, and larynx, or involve the external genitalia. In one instance a transient hemiplegia was thought to be due to edema of the meninges. Dyspnoea and cyanosis rarely occur in this form of serum sickness. During the edematous period there is a reduction in the excretion of chlorides, a gain in weight and some suppression of urine. Moderate albuminuria and cylindruria are not uncommon and occasionally the excretion of phenosulfonphthalein is reduced considerably below normal. The non-protein nitrogen of the blood is rarely elevated. With recovery the renal function returns to normal (72).

Cardiac arrhythmias have been observed in occasional instances (73). Kojis (2 m) collects, from the literature, one case in which transient Stokes-Adams syndrome was associated with serum sickness. Disturbances in the conduction of impulses through the cardiac muscle often accompany anaphylactic shock in cats (74), and rabbits and dogs (75). Though Creip (76) attributed these electrocardiographic changes in rabbits and guinea-pigs to asphyxin, it seems probable from the experiments of Andrus and Wilcox (77) upon the isolated hearts of guinea-pigs and cats, that the disturbance in the mechanism of the heart-beat, during anaphylactic shock, depends directly upon interference with the coronary circulation. They found a decreased blood flow through the coronary arteries in guinea-pigs, which was associated with irregularities such as ventricular extrasystoles, nodal and ventricular tachycardia, prolongation of the P-R interval and complete auricular ventricular dissociation.

Irregularities such as these would be more likely to occur in the immediate and accelerated types of serum disease than in the normal form, but it would not be surprising if, in severe attacks of the usual serum sickness, some changes might be detected in the electrocardiogram; for it has already been pointed out that the acute arteritis, found under such circumstances, may affect the coronary vessels. In one patient with serum sickness, studied by Dr. Andrus, noticeable changes were observed in the T waves. The patient died from the effects of pneumonia. At autopsy Dr. Rich (70) found an acute myocarditis and an acute arteritis affecting the vessels of the kidney, renal pelvis, testes, lung and peripancreatic tissue.

Headache, drowsiness and meningismus may occur in cases with severe reactions accompanied by considerable edema. The cerebrospinal fluid pressure in such cases is increased, and the fluid itself may show elevated globulin and a moderate increase of lymphocytes. Optic neuritis and even choked disc have been observed (78). Kojis (2 m) has found an account of one case in which hemorrhagic encephalitis was discovered at autopsy. The intrathecal administration of antimeningococcus serum has occasionally caused local reactions in the meninges. These may occur with or without the generalized symptoms and signs of serum sickness. An exacerbation of cerebral and spinal symptoms in patients treated with antisera intraspinally is not very uncommon. Lumbar puncture at this time shows increased pressure with an increase of leukocytes. These symptoms have been ascribed to a local serum reaction. Instances have been recorded in which the spinal fluid contained a large proportion of eosinophiles. Pains in the legs and arms often accompany these cerebrospinal reactions.

Symptoms and signs related to involvement of the peripheral nervous system are not extremely uncommon and are of particular interest. For many years the French clinicians have called attention to the occasional occurrence of peripheral neuritis complicating serum disease; and for some time now evidence of involvement of the nerve roots or peripheral nerves has been generally recognized. This phase of serum sickness has been discussed quite fully in recent articles (79, a, b, c, d). The symptoms and signs are classified as spinal, radicular and neural. The cervico-brachial plexus is most often affected with involvement of the fifth and sixth cervical nerves, and thus the upper extremities suffer most frequently. Transient pains of moderate intensity about the shoulder girdle and down one or both arms are not at all uncommon, and usually occur during the late stages of serum disease. Occasionally these symptoms appear earlier, or are of longer duration and of greater intensity, and in a few instances weakness or actual paralysis of the shoulder girdle or arm supervenes. The paralysis is usually described as being of the Erb-Duchene type. The paralysis may persist for weeks or months, but as a rule recovery eventually takes place. Pain, weakness or actual paralysis of the lower limbs is much less frequent.

In addition to these cases in which there is a fairly extensive involvement of the plexus, others are encountered in which single nerves such as the long thoracic, the sciatic or brachial are affected with a resultant neuritis. Mild examples with transient pain and some tenderness are not uncommon, appearing rather as a complication than an actual accompaniment of the disease.

The manner in which the plexuses or single nerves become injured during serum disease is not clear. It is usually thought that edema of the sheath of the nerves or of tissues about the bony ostia might result in pressure upon the nerves. In some respects, however, the symptoms are reminiscent of the peripheral neuritis that accompanies periarteritis nodosa; and in view of the emphasis placed recently upon the arterial lesions in serum sickness, lesions that resemble or actually reproduce periarteritis nodosa, it is possible that the neuritis may depend upon similar arterial changes in the nerves or in their sheaths.

Serum disease is accompanied by a well marked leucocytosis with increase, at first, of the polymorphonuclear cells. In children, particularly, as pointed out by Pirquet and Schick, a leukopenia with increase in mononuclear cells may follow the leucocytosis. Eosinophilia is not characteristic of the disease.

The description so far has been of the form of serum sickness which occurs in individuals who have not previously been treated with a specific foreign protein, or who have not spontaneously acquired sensitivity to it. If either of these conditions exists, the injection of the same protein results either in an accelerated reaction or an immediate reaction. The accelerated reactions appear within one to three days after the administration of the antisera and differ from the ordinary serum disease in that the incubation period is shorter, the onset more explosive, the symptoms more violent and the course frequently shorter. Acute collapse with fall in blood pressure is not unusual, and respiratory difficulty with asthma is observed. Frequently the acute oedema is alarming. These symptoms may occur when a second dose of serum is given from three to four weeks after the primary inoculation to several years after the first dose.

The immediate reactions are the most serious ones and are particularly likely to produce violent, alarming and even fatal reactions in persons, such as asthmatics, who are naturally sensitive to the particular protein employed. These reactions must be differentiated from the immediate febrile or anaphylactoid shock which may come on immediately, or within a few hours, after the intravenous injection of a variety of substances (80). Sudden dyspnoea, cyanosis asphyxia, generalized edema, circulatory collapse, convulsion or profuse urticaria, are the most common symptoms and signs. The condition, in fact, is an acute anaphylactic shock. Death may be almost instantaneous or occur only after several hours. Kojis (2 m) has collected accounts of 61 deaths following the injection of foreign serum. This may occur when a second injection of the specific foreign serum is given from twelve days to ten years after the first. Sixteen of these fatal cases had had asthma or were afflicted with chronic respiratory diseases. Thirteen had had previous injections of serum. The immediate reactions fulminate most frequently when the serum is administered intravenously, though death has followed even an intradermal injection of such small amounts of serum as 0.1 to 0.05 cc.

It is now interesting to compare certain types of drug reactions with the clinical picture of classical serum disease. There are three groups of drugs that produce a variety of sickness simulating in many respects serum sickness; these are nirvanol (the sodium salt of phenyl-ethyl-hydantoin), the group of arsphenamines and the sulfonamides.

Nirvanol sickness is a well defined disease that has been repeatedly observed owing to the fact that the drug was employed a few years ago fairly extensively for the treatment of Sydenham's chorea (81, a, b, c). It was believed by some that its therapeutic value depended upon this characteristic reaction. Unlike the reactions caused by many other drugs, Nirvanol produces with remarkable regularity, in all patients, exactly the same symptoms and signs, with much the same incubation period; so that "Nirvanol sickness" is a disease as well defined as measles or chickenpox.

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mately nine days after the first injection of neoarsphenamine is an extremely interesting illness which has recently been extensively discussed and analyzed by Peters (83). Amongst the numerous intoxications resulting from arsenotherapy (84, 85) it resembles more closely than any other serum sickness. It may follow the administration of almost any one of the antisyphilitic arsenicals, and has quite a constant incubation period varying from 5 to 19 days but averaging 7 to 8 days. As a rule one to three injections of the arsenical intervene between the first dose and the onset of the illness. The sickness starts abruptly with malaise, chills, fever, anorexia, nausea, vomiting, generalized aches, headache and sore throat. A rash appears on the second day. This may be morbilliform, scarlantiniform, macular, multiforme or occasionally urticarial. There is often suffusion of the conjunctivae. The majority of patients have general enlargement of lymph nodes (70% Peters). Enlargement of the liver and spleen may occur, and jaundice is not unusual. In about one-half of the cases the leucocyte count is not increased, and there may be actual leucopenia, occasionally with agranulocytosis. In other patients there is a leucocytosis. Almost half of the cases show a moderate or pronounced eosinophilia. Nephritis and hemorrhagic encephalitis may occur as complications. The disease lasts for an average of 6 days.

Subsequent treatment with arsenicals within two weeks of this syndrome is followed by serious complications, and a recurrence of the fever and rash may appear immediately after the reinstitution of treatment by the arsenical. This is illustrated in the accompanying chart (Chart IV). Even when reinstitution of treatment is postponed for weeks or months, following the attack of erythema of the ninth day, various forms of arsenical intoxication may result in about one-fourth of the patients. Among these may be a recurrence of the typical erythema of the ninth day.

Although the disease is best explained as a sensitization phenomenon, closely resembling serum disease, it has been almost impossible to obtain definite evidence of specific sensitization in these patients. The patch test to arsenicals and related compounds has rarely been found positive; and the transfer of passive sensitization by the Prausnitz-Küstner method has also been reported as negative.

The literature upon the toxic reactions to the sulfonamide group of drugs has reached extensive proportions (86, a, b, c, d, e). These reactions are extremely varied, differ somewhat with the form of sulfonamide employed and are undoubtedly due to different causes.

The commonest early unpleasant effects following the administration of the sulfonamides are nausea, vomiting, cyanosis, headache, dizziness, mental confusion and acidosis (sulfanilamide). These symptoms are probably to be ascribed to an intoxication by the drug, and should at present be considered as such. The more serious results consisting among others of fever, eruptions, profound anemia, jaundice and agranulocytosis may well be of a different nature.

Hageman and Blake (87) first called attention to certain specific reactions

which occurred in 15.6% of 134 cases treated with sulfanilamide. Symptoms appeared between the fourth and thirteenth day of treatment. In 10 of their 21 patients the first symptoms were noted on the seventh and eighth days. In the majority of patients there was an abrupt onset of fever with malaise, nausea, vomiting, itching and tinnitus. In nine instances a maculopapular erythema occurred, while hemorrhagic lesions and urticaria like wheals were noted in a few of these. Hepatitis with jaundice and stupor occurred in one patient. The

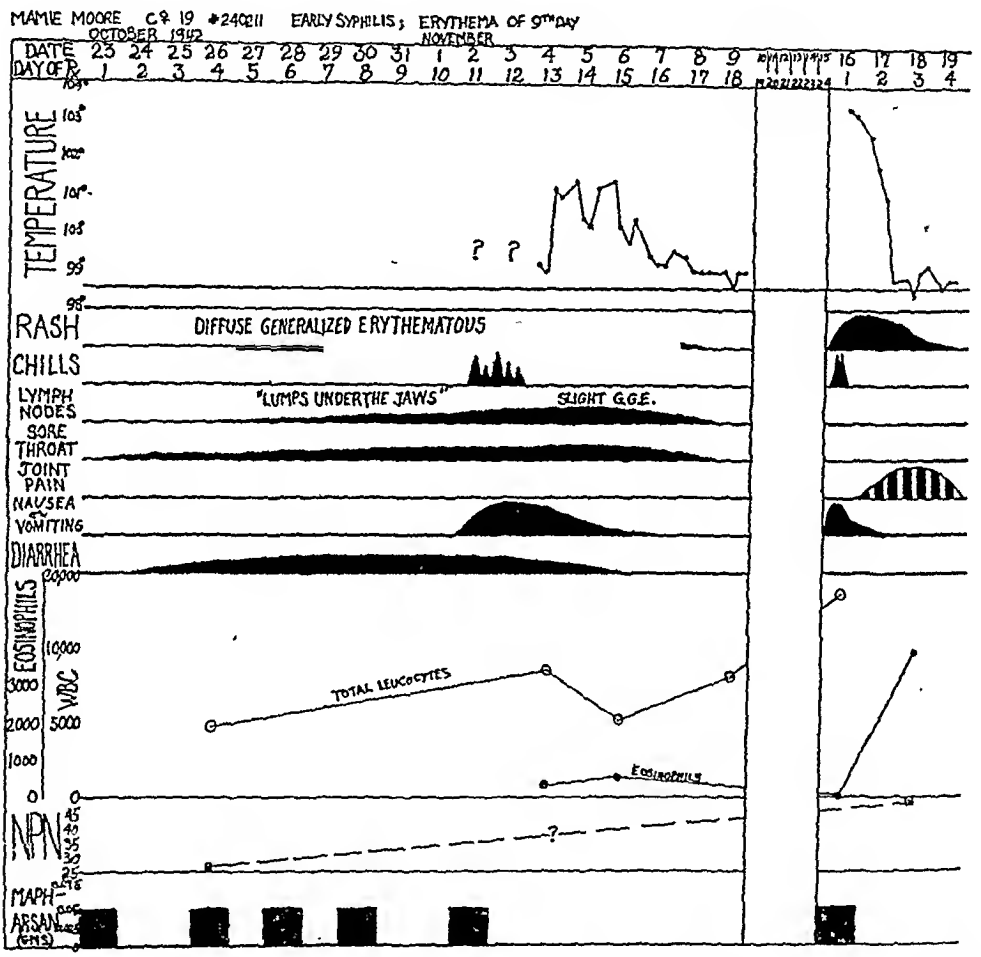

CHART IV. Illustrates the clinical course of "Erythema of the ninth day" following injections of Mapharsan. The reaction after the first course of Mapharsan is of the "normal" type. The reaction after the first dose of the second course is of the "accelerated" type.

general reaction appeared in four patients from one to five days after the drug had been stopped; in others the reaction seemed to be prolonged by a continuation of the drug. There was frequently a leucocytosis, with some increase in the percentage of eosinophiles. The average duration of the fever was from two to four days.

Since this publication a vast amount of literature has accumulated on the subject of toxic reactions following the use of all the sulfonamide drugs. In general they fall into three catagories: first, the acute haemorrhagic anaemia;

secondly, agranulocytosis; and thirdly, the febrile constitutional reactions accompanied in most instances by cutaneous eruptions.

The acute haemolytic anaemia, first described by Harvey and Janeway (88) as a complication of sulfanilamide therapy, has been observed repeatedly, and occurs particularly after the use of sulfanilamide and sulfapyridine. The first evidences of anaemia appear early, sometimes on the first day of therapy, and almost always before the fifth day. The anaemia may develop rapidly and may be profound; but when the drug is stopped and transfusions of blood are instituted, recovery takes place fairly promptly. A second course of the sulfonamide drug may precipitate another acute attack of haemolytic anaemia.

The reaction is analogous to the immediate or accelerated form of serum sickness which occurs in a person previously sensitized to the specific protein employed. In this connection it is interesting to note that Salvin (89) has reported an acute anaphylactic reaction characterized by sneezing, lacrimation, shortness of breath, urticaria and fever that appeared within 24 hours after the first dose of sulfanilamide. Some days after recovery a similar acute reaction was precipitated by administering a single dose of 0.065 grams of sulfanilamide. It is therefore possible that an individual may become spontaneously sensitive to sulfanilamide or to one of the radicals that go to form this drug.

The acute agranulocytosis occurs almost exclusively after the use of sulfanilamide and sulfapyridine. Findland (90) and his associates did not encounter a single instance of agranulocytosis in 446 cases of pneumonia treated by sulfadiazine. The first symptoms appear late, after the prolonged use of large amounts of the drug. The usual time of their occurrence is between the 14th and 21st day after the drug has been started. The onset is usually accompanied by fever, sore throat, stomatitis and a skin eruption. The disease is apt to run a rapid course and the mortality is high, death supervening in 70% of the reported cases (86 c). Leukopenia, which is not an uncommon accompaniment of the intoxications from all of the sulfonamide drugs, often precedes the agranulocytosis.

The third great group of cases, which is much the largest, includes patients treated with any one of the sulfonamides. The reactions are characterized by a multiplicity of symptoms and signs, some of which are more common after the use of one compound, some after another (Chart V).

Fever and skin eruptions are the most frequent evidences of intoxication, and may be encountered after the use of any one of the sulfonamides. The fever may be transient or last for days. The eruptions are extremely varied and range from mild erythema to generalized exfoliative dermatitis. Urticaria and purpura are sometimes seen, and erythema nodosum, conjunctivitis and episcleritis result particularly from the use of sulfathiazole (91). Jaundice, hepatitis, cardiac arrhythmia (92), painful joints, oedema, marked lymphadenopathy, optic neuritis (93), peripheral neuritis (91 n), and coma with encephalitis[1] have all been recorded, though they are unusual, and are said to occur only after one or another of the sulfonamides.

The renal complications are of particular interest and of great importance.

[1] Personal observation.

They are described as complicating the use of sulfapyridine, sulfathiazole and sulfadiazine. In addition to the symptoms and signs of urinary calculus, which result from the precipitation of the acetylated form of these drugs in the urinary tract, and which are of mechanical nature, there may develop a serious form of renal insufficiency.

In the presence usually of fever, eruptions or other symptoms, oliguria develops. The urine is of low specific gravity and contains varying amounts of albumin and casts. Hematuria is frequent. With the appearance of oliguria, which may progress to anuria, the non protein nitrogen of the blood mounts, the excretion of phenosulphonphthalein falls and the urea clearance diminishes to very low levels. When the drug is stopped, and large amounts of fluid administered, recovery from the acute attack takes place in the majority of cases,

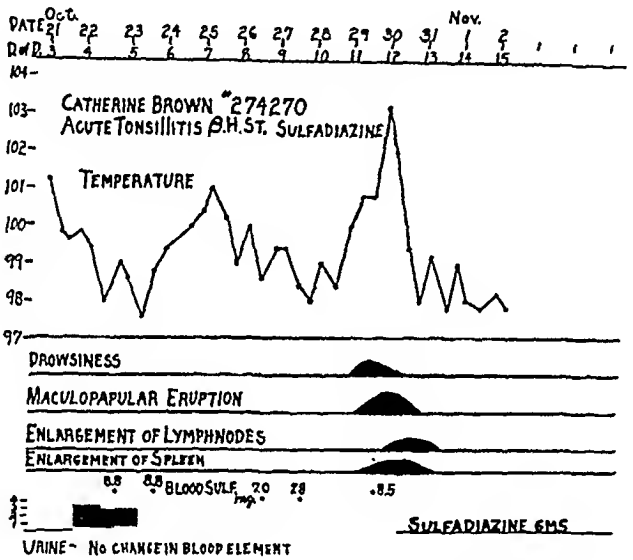

CHART V. Illustrating a mild generalized reaction to sulfadiazine, appearing on the 7th day after the first dose of sulfadiazine

and permanent restitution to normal may result. When anuria is not relieved death may ensue.

Involvement of the kidney is almost unknown after the use of sulfanilamide, but the following case shows that this may occur when it follows the administration of sulfathiazole in the presence of an acute hemorrhagic nephritis.

C. A., Male, colored, (Unit 243276), aged 29, was admitted to the Johns Hopkins Hospital on Jan. 26, 1942, complaining of shortness of breath with swelling of the face and body of ten days duration.

These symptoms appeared suddenly, and, on account of difficulty in urination, he went to the urological dispensary. He was catheterized and grossly bloody urine was obtained. On Jan. 13 he was given sulfathiazole. He received 4 grams a day from Jan. 13 to Jan. 15, amounting in all to 12 grams over these three days. He had anorexia and vomiting, developed increasing shortness of breath and generalized oedema and was admitted to the hospital on Jan. 26 with anasarca, hypertension, rales at the bases of the lungs, albuminuria, haematuria and cylindruria. The heart was enlarged; the blood pressure elevated, and the liver was palpable. The Wassermann reaction was negative. One week before the onset

of his illness he had run a splinter into his finger which had become infected and discharged pus. The wound had healed, but the throat, which did not show evidence of acute infection, furnished, nevertheless, on culture haemolytic streptococci. It was evident that the patient was suffering from a severe attack of acute haemorrhagic nephritis, following an infection of the finger. Since he was still carrying haemolytic streptococci in the throat he was placed on prophylactic doses of sulfanilamide (Charts VI and VII).

It is impossible to say whether the toxic reaction was due to sulfathiazole, sulfanilamide or a combination of the two, but it is probable that the renal insufficiency occasioned by the

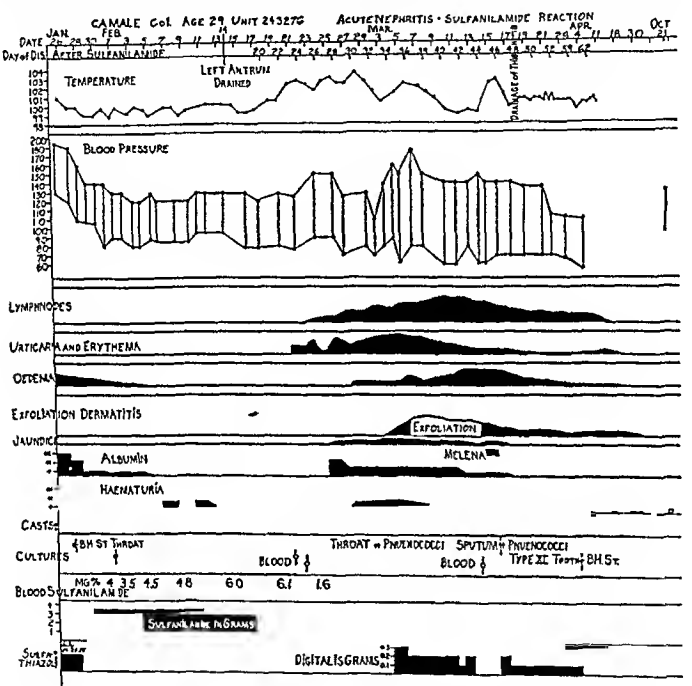

CHART VI. Illustrating the clinical course of a severe reaction following a course of sulfanilamide preceded by a short course of sulfathiazole

acute nephritis was responsible for a retarded excretion of sulfanilamide and possibly of sulfathiazole.

Complete recovery ensued both from the intoxication and from the acute nephritis and on Oct. 12, 1942, the patient was hard at work, in excellent health, and the physical examination showed no abnormalities. The renal function, including the phthalein excretion, the non protein nitrogen of the blood, and the urea clearance were normal.

In addition to the chart, showing the course of the illness in this patient, there is appended a table giving the figures for remarkable alterations in blood chemistry. It can be seen that during the period of jaundice there was a retention of bromsulphonphthalein and increase in units of phosphatase.

The acute intoxication in this patient presented many of the characteristics of serum sickness with several features that are common in drug reactions, but that are absent in typical serum disease. Among these were the progress of the eruption to exfoliative dermatitis, the noticeable eosinophilia and the marked acute renal insufficiency with azotemia and hyperchloraemia.

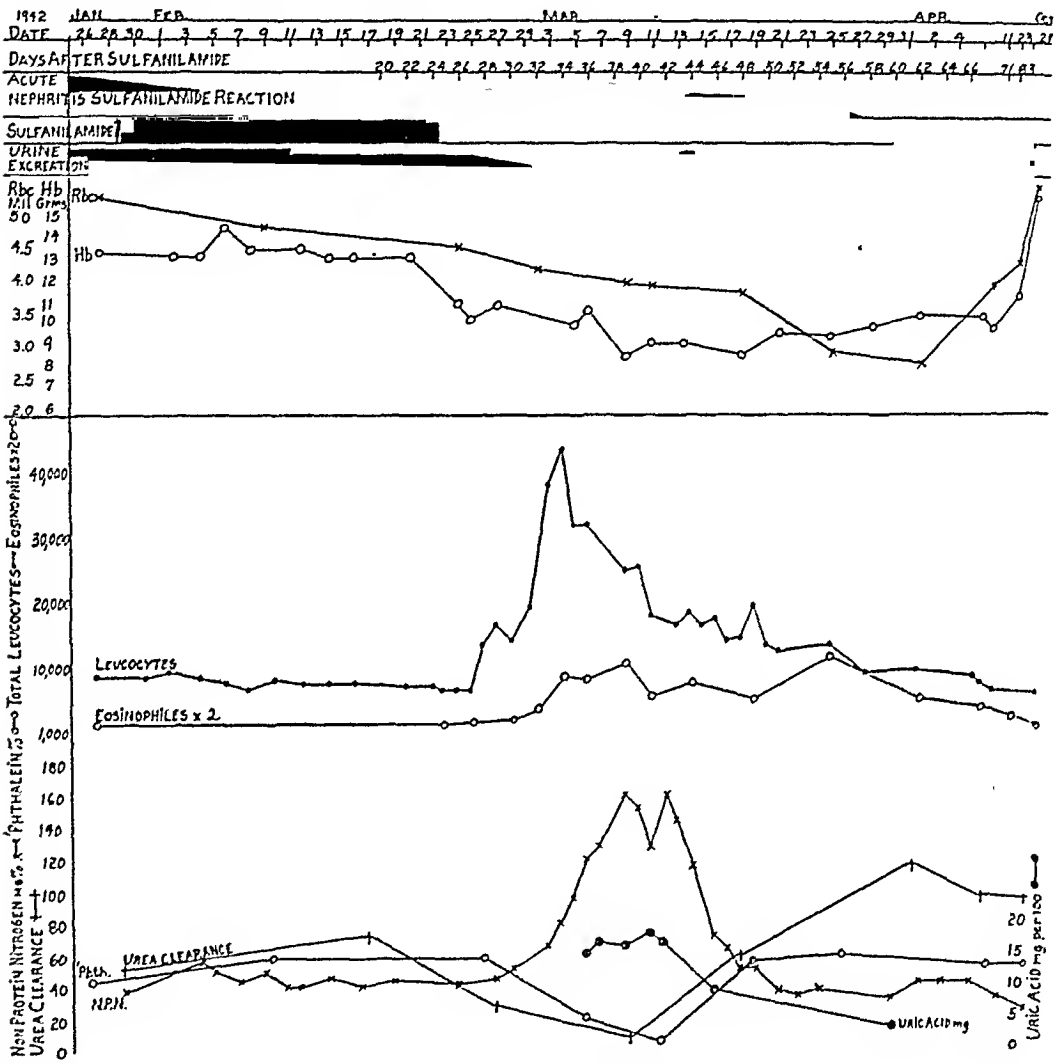

CHART VII. Illustrating the changes in the formed elements and chemistry of the blood in the same patient as in Chart VI

As information accumulates, there is increasing evidence to indicate that these toxic reactions to the sulfonamides are expressions of an allergic response to the drugs. These generalized reactions with fever, dermatitis and involvement of various systems usually appear from 7 to 9 days after the drug has been started. In a few instances symptoms occur during the first 24 or 48 hours and may be truly anaphylactic in character (89). The immediate or accelerated reactions are, however, usually observed in patients who have had a previous course of the drug. In other instances the reaction is delayed and does not appear for 14 to 21 days or longer. There are many reports to show that one course of sulfanil-

amide or sulfathiazole predisposes the patient, later on, to an acute immediate reaction upon administering the first dose of the same drug. An eruption may start suddenly within a few minutes after the drug is taken (95, a, b). Elevations of fever may follow within an hour of the first dose of a second course of sulfathiazole. Lyons and Balberor (96) state that 19 of 53 patients, to whom

TABLE 1

Conrad Amos

DATE	CO_2	Na	Cl	PHOSPHORUS	CHOLESTEROL	VAN DEN BERGH	BROMSULPHTH.	PHOSPHATASE
	vols %	*m eq.*	*m eq.*	*mmg per 100 cc.*	*mmg. per 100 cc*	*units*	*% retention*	*units*
1-27	56		101.2		144			
2- 6	39		111.8					
2-11	35.3							
2-13	39		111.2					
2-14	42.8		110.2					
2-17	50.4		119.4					
2-19	36.2		110.0					
2-24	42.8		105.8					
2-25						0.7 biph.		
2-27	52.2		10.2					
						2.8 biph.		
3- 2				5.2		4.0 biph.	50%	10.8
3- 3					125	2.1 biph.		
3- 4	43.8		89.2					
3- 5	41.9		84.6			2.8 biph.		
3- 6	49.4		85.6					
3- 7	39.0		83.4			2.7 biph.		
3- 9	43.8		89.0			1.7 biph.		
3-10	43.8		92.8					
3-11	41.9		94.0					
3-12	61.7		102.8	11.5		0.6 biph.		19.2
3-13	51.3		101.8					
3-16	52.2		105.0					
3-17				6.0				8.6
3-19	50.4		108.8					
3-23	46.6		112.4					
3-25	48.5	126.2	107.0					
3-26	46.6	133.1	108.8					
3-30	49.4		111.4					
4- 1	50.4		108.0			?	ft. trace	4.5
4- 7	49.4		107.2					
4-13	50.4		104.6					

sulfathiazole was readministered, responded by an immediate elevation of temperature. Similar results have been recorded by Nelson (97), who has found, in addition, that the acquired sensitivity to sulfathiazole in his patient was not limited to this drug; since an acute febrile reaction also followed the administration of sulfadiazine and sulfapyridine. We have recently seen a pregnant

woman who developed an alarming reaction following the first dose of a second course of sulfathiazole. On account of a suspected infection shortly before delivery she was given a short course of sulfathiazole (35 grams in 5 days). The drug was stopped after delivery but 5 days later, because of slight elevation of temperature, a second course of sulfathiazole was instituted. About two hours after she had taken the first dose of 2 grams of sulfathiazole, there was a rapid rise in temperature to 104° with delirium. Two days later the temperature was 105.8 and she was in coma. The drug was stopped after 2 days, but the patient remained febrile and developed signs of encephalitis.

The changes referable to the nervous system and to the neuritis particularly, though rare, deserve special mention (98); for Rich (70) has found an acute arteritis, presenting the histological picture of periarteritis nodosa, to be present in both serum disease and death during sulfonamide intoxication. In view of this fact it is interesting to point out that peripheral neuritis forms one of the fairly common symptoms of typical instances of periarteritis nodosa.

In spite of the circumstances that have led many to regard these intoxications as a form of allergy to one or another of the sulfonamide drugs, it has been almost impossible to bring proof of this by the use of the conventional tests. Occasionally the patch skin test is positive (89), but this is rare, and other specific reactions cannot be demonstrated. The situation is quite the same as that which exists in other forms of drug allergy.

The entire subject of sensitization to chemical substances is quite complex, as has been pointed out in an earlier part of this discussion, and it is known that in the case of some compounds (Landsteiner, Sulzburger) the antigen consists, not of the complete chemical substance, but of a single radical which, either alone or more often in conjugation with the proteins of the body, forms a complete antigen. It is noteworthy that Davis (27, a, b) found each one of the sulfonamides with which he worked would combine in vitro with the human serum in different proportions. This indicates that the affinity for the protein varied with the drug employed. Since each of the sulfonamides contains specific radicles as well as a common radicle, it is conceivable that the patient is not sensitized to the entire drug, but may be sensitized to a single radicle of the chemical compound which conjugates with body proteins. For instance, in sulfanilamide intoxication, the haemolytic anaemia might be caused by sensitization to one radicle, the agranulocytosis to another, while the great mass of reactions occurring usually after an incubation of 7 to 9 days might be an evidence of sensitization to one or more radicles common to the entire group. The situation might be somewhat analogous to the condition that exists in normal horse serum, and the serum obtained from immunized horses.

Finally the limited information obtained at the present time from the study of autopsy material is important. Several cases of sulfathiazole poisoning have been reported with death and autopsy (99, a, b, c). The characteristic lesion in these cases consisted of minute foci of necrosis with cellular infiltration scattered throughout the organs. This was combined with cellular degenerations.

One case of fatal anuria following sulfadiazine therapy has been reported by Hellwig and Reed (100). Focal necroses were found in the liver with severe degeneration of the convoluted tubules and ascending loops of Henle in the kidney. In the dog extensive lesions of blood vessels have been produced by subcutaneous injections of sulfadiazine (100a). Perhaps the most important observations are those of Rich (70), already referred to, who described a form of periarteritis nodosa affecting the small vessels of many organs as the characteristic pathological lesion in sulfonamide intoxication as well as in serum disease.

After consideration of this entire problem it seems justifiable to conclude that many so-called toxic reactions following the administration of the sulfonamides, as well as some other chemical drugs, are analogous to serum disease, and are due to a sensitization to the chemical or to one of the radicles of which it is composed. These intoxications appear in the form of the normal serum sickness, the accelerated serum sickness and the immediate serum reaction.

DIAGNOSIS AND TREATMENT

When serum disease occurs as a consequence of a prophylactic dose of antiserum, it is usually recognized, at onset, without difficulty. There is one symptom, however, which may occur under circumstances that alarm both patient and doctor. Stiffness of the jaws may be the first symptom to appear in an individual who has received a prophylactic dose of tetanus antitoxin. This results in great anxiety, which is only relieved by the sudden appearance of an urticarial eruption.

On the other hand, it may be extremely difficult to distinguish serum sickness from the onset of some complication when antiserum is used for the treatment of pneumonia or scarlet fever. There is only one sign of the disease that is pathognomonic, and that is urticaria. Every other symptom or sign may occur as a complication of the acute infection itself—fever, leucocytosis and arthritis may all usher in a complication of pneumonia. Fever, secondary rashes, lymphadenitis and arthritis may occur as complications or exacerbations of scarlet fever. A mild rheumatic fever, complicating scarlatina, is readily confused with serum disease. When the sulfonamide drugs are employed in combination with antisera, the situation presents even more of a problem; for the physician must determine whether he is dealing with a complication of the disease, an intoxication from the drug which appears as a special form of serum sickness, or with serum disease itself.

There are no specific means of detecting serum disease in its early stages. The appearance and concentration of heterophile antibodies for sheep cells is very irregular. The specific precipitins do not usually appear in the blood until the latter part of serum disease, and the same applies to the Prausnitz-Küstner reaction and the development of skin sensitivity. One must, therefore, rely upon the development of symptoms and signs at a time which is commensurate with that usual for the onset of serum sickness.

The Prevention and Treatment of Serum Sickness

Preparation of antigen. The usual method of preparing antisera at the present time is to concentrate the antibody and antitoxin in as small an amount of protein as possible. This has great advantages since it reduces very materially the bulk of foreign protein injected; but it so happens that these antibodies and antitoxins are associated with or contained in the globulin fraction, and it is just this protein that is most highly antigenic. Serum disease seems, therefore, to be almost as common, though it may not be as severe, following the administration of concentrated antisera as following the administration of whole serum.

Attempts have been made to reduce in one way or another the antigenic property of these sera. Heat, acidification or alkalinization, peptic digestion, and the yeast ferment takidiastase have been used, but these methods, with the possible exception of peptic digestion, give rise to immunological changes which show a deviation in specificity accompanying the reduction in antigenic activity of the treated product. Kass, Sherago and Weaver (101) have recently reviewed this entire subject and report the results of their experiments with diastases and pepsin on antitoxic and normal horse plasmas. They find that animals sensitized to undigested normal or undigested antitoxic plasmas do not react to digested antitoxic nor to digested normal plasmas. On the other hand, animals sensitized to unheated digested plasmas react not only to the homologous antigens but also to undigested plasmas. More complicated differences were observed if antitoxic plasmas were subjected to digestion and then to heat.

Some experiments dealing with the effect of photo-oxidation upon antigens and antibodies are of particular interest. Smetana and Shemin (102) have studied the property of antigens and antibodies which have first been sensitized by the addition of HCl hematoporphyryn and then exposed to visible light (103). They discovered from the electrophoretic studies of egg albumin, antisera and their specific antibody fractions that photo-oxidation caused a marked alteration in the pattern of these substrates. The alterations produced by photo-oxidation were associated with a progressive destruction of the antigenic function of egg albumin and with a progressive lowering of the precipitating power of anti-egg albumin rabbit serum and antipneumococcus type I horse serum. Egg albumin whose precipitin reaction is destroyed by photo-oxidation no longer causes anaphylaxis in guinea pigs and does not produce precipitins in rabbits. Henry (104) has carried these observations much further; for he has investigated not only the effect of visible light but of ultraviolet light upon the antigenic property of normal horse serum. The sera were treated with hematoporphyryn, exposed to the air in thin layers and agitated during irradiation. Visible light acting in combination with hematoporphyryn for four 6 hourly doses, caused almost complete extinction of the precipitin reaction, and sera failed to produce anaphylactic shock in guinea pigs fully sensitized to horse serum. Animals sensitized to the irradiated sera, however, reacted to normal horse serum. This indicated that sufficient unchanged horse serum antigen remained after irradiation to sensitize guinea pigs. Irradiation with ultraviolet light and hematoporphyryn proved much more effective. The treatment of normal horse serum in this

manner for 96 hours resulted in a product which did not sensitize guinea pigs after three intraperitoneal injections of 0.1 mg. of protein at the end of 3 to 4 weeks, since a shocking dose of 4 cc. of this irradiated serum failed to produce anaphylactic shock. The implication was that the antigenicity of this serum was of the order of 1–10,000 that of normal serum, and that the residual content of antigen having the specificity of normal horse serum may be as low as $\frac{1}{100}$ cc. of horse serum per liter.

Pending the further development of methods to reduce or abolish the antigenic property of foreign sera, it remains of utmost importance to question the patient most carefully, or to test his sensitivity to the particular foreign serum before this serum is employed for therapeutic purposes. The intracutaneous test, the conjunctival test and intravenous test should all be employed. Whole horse serum is irritating when injected intravenously or when it is introduced into the conjunctival sac and therefore proper dilutions of serum must be selected. The intracutaneous test may be performed with 0.02 cc. of 1–10 or 1–100 dilution of serum, and at the same time a control test should be made with physiological salt solution. For the conjunctival test one drop of a 1–100 dilution of serum should be employed. If there is no reaction to this test after one-half to one hour, the final intravenous test may be performed, which is carried out by the intravenous injection of 0.1 cc. or less of the therapeutic antiserum. If again the test proves negative, treatment may be carried out in the usual manner. The intravenous test should never be neglected for it occasionally happens that the patient, though sensitive to the particular serum, gives both a negative intracutaneous and conjunctival test.

Should these latter tests be positive, it becomes essential to desensitize the patient before serum is administered. This is rarely possible to carry out with complete success, but the sensitivity of some patients may be so much reduced that it is possible to administer serum in accord with the therapeutic needs, without producing more serious results than generalized urticaria or slight asthma as the doses of serum are increased in amount (2 i, 105).

The treatment of serum disease, once it appears, is extremely unsatisfactory. Derick, Hitchcock and Swift (106) thought that the arthralgia could be prevented or diminished in severity by continuous administration of salicylate or aspirin during the incubation period. These drugs, however, had no effect upon the other symptoms. Subcutaneous injections of adrenalin will relieve temporarily the unbearable itching of the urticaria. Sedation may be required to relieve the pain of arthralgia.

CONCLUSIONS

In conclusion, serum disease and the related phenomena must be regarded as an outward and visible sign of the process of sensitization of the human being to a foreign protein, and is accompanied and followed by the immunological reactions which occur commonly in experimental anaphylaxis in animals. A similar disease is observed after the administration of certain drugs, such as Nirvanol, the arsphenamines, and the sulfonamides. They produce systemic

reactions analogous to serum sickness in its normal, accelerated and immediate forms; but these differ from serum disease in the particular that specific antibodies have not been demonstrated so far in the serum, and that skin reactions to the specific drug are rarely obtained. Efforts to obviate serum disease following the administration of foreign serum are being directed towards the use of a purified albumin fraction from bovine serum, and in an attempt to render normal heterologous serum non-antigenic by various physical and chemical devices. So far these experiments have not been completely successful.

There is great danger in administering foreign proteins to patients who have natural or acquired sensitivity to these specific proteins. Methods are available to detect such sensitization and therefore precaution should be taken to prevent these unfortunate accidents. Desensitization is difficult and the treatment of serum disease is extremely unsatisfactory.

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ERRATUM

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THE IRIS

Innervation of the Iris of the Albino Rabbit as Related to Its Function. Theoretical Discussion of Abnormalities of the Pupils Observed in Man

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Part I. Review of the Literature Dealing with the Structure and Innervation of the Iris and Responses of the Pupil

INTRODUCTION

Whenever organs in the body are innervated by both sympathetic and parasympathetic fibers of the autonomic nervous system, considerable interest arises as to the exact structures supplied by each of these two groups of nerves and the functional relationship of one to the other. The theory has often been advanced when these two groups of nerve fibers innervate an organ, that they control functions which are antagonistic. Thus sympathetic fibers initiate dilatation of the pupil and parasympathetic fibers, constriction. With further experimental work, it appears that this rule does not generally hold, and there is considerable doubt at the present time as to the antagonism in function of these two systems of autonomic fibers. However, the part which each of them plays has never been adequately defined, either from an anatomical or a physiological point of view.

The work which is reported here was begun in the hope of elucidating further this whole problem, for there is no example of a structure in which the antagonism of the two groups of fibers has been more widely accepted than the iris. It is usually stated that the parasympathetic fibers reaching the iris by way of the oculomotor nerve and the ciliary ganglion supply the sphincter muscle of the iris and by producing its contraction bring about a constriction of the pupil. On the other hand, the sympathetic fibers, with their relay station in the superior cervical ganglion, are believed to innervate a dilator muscle of the iris which by its contraction tends to enlarge the pupil. A correlation between the two balanced systems, therefore, would determine the size of the pupil at any particular moment. If this theory is accepted, it presents an admirable example of the balanced functions of the two fiber systems. Experimental work reported here is related to this whole problem.

In support of the idea that the sympathetic and parasympathetic fibers innervate different mechanisms which have an opposing effect upon the size of the pupil of the eye, the following positive findings must immediately be taken into consideration: Stimulation of the oculomotor nerve produces a constriction of the pupil and section of this nerve causes the pupil to dilate to a maximal degree. On the other hand, stimulation of the sympathetic fibers causes the pupil to

dilate and section of these fibers gives rise to a constriction of the pupil so that it becomes somewhat smaller than the one upon the opposite side. However, the constriction which follows section of the sympathetic pathway is by no means a maximal one.

This control by two systems of nerve fibers of opposite functions in an organ composed of smooth muscle would differ from the innervation of striated muscle. In the latter case, one group of nerves can influence the muscle fibers either to greater or less tone and contraction.

It might be assumed in the pupil that four possible factors must be taken into consideration. Increased or decreased stimuli along parasympathetic pathways diminish or enlarge the diameter of the pupil, and the same is true of the sympathetic pathway. To make this matter clearer, it is important to consider the reflex pathways influencing the size of the pupil. Light influences pupillary diameter purely through the parasympathetic fibers. Indeed, pain appears to be the only type of sensory stimulus which has been considered to modify the pupil through the mediation of sympathetic nerves.

A detailed study of this problem of dual innervation as related to another organ of the body, the urinary bladder, has already been made (Langworthy, Kolb and Lewis, 1940). The findings were tested and applied to patients suffering from injuries to the peripheral or central innervation of the bladder mechanism. As a result of these observations, it was concluded that the parasympathetic portion of the vesical innervation was of paramount importance in bladder functions both of storage and expulsion of urine. The sympathetic component of the autonomic nervous system appeared to control the vascular supply of the bladder and to be of only minor importance in the fundamental activity of the bladder itself.

SUMMARY OF RECENT PHYSIOLOGICAL EXPERIMENTS PERTAINING TO THE INNERVATION OF THE IRIS

The need for a dilator muscle acting as antagonist to the sphincter of the iris was not felt until the end of the nineteenth century. Anatomists have never clearly demonstrated an adequate group of muscle fibers to serve as a dilator muscle in the iris. Recent work has shown that reflex changes producing either constriction or dilatation of the pupil are controlled, chiefly if not entirely, through the parasympathetic innervation acting upon the sphincter muscle of the iris. The information given here dealing with early experiments concerned with the anatomy and physiology of the iris was obtained largely from a thesis by A. Magitot, called "L'Iris" published in Paris in 1921. References to the earlier articles will be found in this Paris thesis.

Anatomists and physiologists were first content to explain the movements of the pupil by turgescence of the spongy vascular tissue of the iris. It was noticed that the pupil responded with small oscillations to the pulse, and assumed that dilatation and constriction of the vascular bed had an important influence on the pupil. The movement of the iris was thought to be due to the vasomotor action of the nerves. Contraction of the pupil was related to dilatation of the

vessels and dilatation to their contraction. A floating membrane, such as the iris, shows a strong response to the pulse. Claude Bernard in 1852 demonstrated that excitation of the cervical sympathetic trunks produced a pallor of the iris and conjunctivae. Budge and Waller (1852) judged that the movements of the iris were the result of vascular contraction and dilatation. This opinion was supported by anatomists, who found that injection of the blood vessels caused a constriction of the pupil.

Schiff in 1872 suggested that two types of fibers exist in the cervical sympathetic trunk, vasomotor to the blood vessels and dilators of the pupil. François-Franck believed that these two groups could be separated in the carotid plexus and stimulated individually.

When the constrictor muscle was discovered, its contraction and relaxation were used to explain the movements of the pupil. Many workers found this explanation insufficient. The old ideas appeared under a new form and an important role in dilatation of the pupil was attributed to the blood vessels.

Langley and Anderson (1892) on the basis of rather obscure arguments, many of which are no longer considered valid, felt a necessity for a distinct dilator muscle of the iris. They found in the cat that stimulation of the cervical sympathetic trunk with a weak current produced a prompt dilatation of the pupil and a slow contraction of the arteries. They believed that no increase of any importance in the radial tension of the iris could be caused by contraction of its blood vessels or by relaxation of the ciliary muscle. When the sclerotic coat was stimulated after all the ciliary nerves, except one long ciliary (sympathetic fibers), were cut, the part of the iris opposite the stimulus was dragged over to the stimulated side. These investigators believed that it was impossible to account for these facts on the theory that the movements of the iris were produced solely by a sphincter muscle capable of contraction and relaxation and by radial elastic fibers. They found that a dilatation of the pupil may occur in a part of the iris where the sphincter is strongly contracted. It followed that the dilatation of the pupil could not be caused by an inhibition or a relaxation of the tone of the sphincter. Other experiments seemed to show that the sympathetic fibers have no inhibitory power upon the sphincter muscle. However, Langley and Anderson did not satisfy themselves that the radial contractile element had the form of ordinary unstriated muscular tissue.

Langley and Anderson summarized the previous theories concerning pupillary dilatation as follows: First, the dilatation may be caused by decreased blood in the iris, longitudinal contraction of the radial arteries or of the longitudinal muscle in the arteries. Second, dilatation may be due to radially arranged muscle fibers. Third, inhibition of the sphincter muscle may cause a dilatation. This implies an elastic tissue in the iris which is more or less stretched, except when the pupil dilates to its fullest extent. Fourth, relaxation of the ciliary muscle may pull the iris backward leading to dilatation of the pupil. This implies an elasticity on the part of the ciliary region in the anterior part of the choroid which would come into play and pull the iris backward. The fifth possibility was a combination of these different causes.

Certain other arguments were advanced against the function of the blood vessels controlling dilatation of the pupil. Thus when the pupil was distended with atropine, the blood vessels became tortuous but not constricted. At the same time, stimulation of the sympathetic trunk produced a further dilatation. It must be remembered, of course, that atropine produces dilatation of the pupil by causing a paralysis of the sphincter muscle but has no effect on the blood vessels. Similarly, it was found that if the pupil was made to constrict by the injection of eserine, stimulation of the sympathetic fibers had no effect. It produced pallor of the iris, but no pupillary dilatation. Under these conditions, it was probably impossible to overcome the spasm of the sphincter muscle. Moreover, recent studies prove that this last argument does not hold, inasmuch as it has been shown that dilatation can be induced reflexly when the pupil is constricted by eserine.

It must be realized that dilatation of the pupil has never been clearly related to a specific muscle. Several opinions have been given concerning the possibility of a dilator muscle. One group of anatomists thought that there was no dilator muscle. Another group felt that the dilator was made up of radial smooth muscle fibers which were distinct, one from the other and situated in the posterior part of the stroma of the iris. A third group believed that the dilator was represented by a muscular membrane, which lies on the posterior surface of the stroma, just anterior to the inner layer of pigmented epithelium which is described as a posterior limiting membrane.

Toward the end of the last century, a long series of articles were published concerning the possible location of a dilator muscle. The large group of workers who denied the existence of any dilator muscle fibers still attributed importance to the blood vessels for the mechanism of pupillary dilatation.

A number of German anatomists led by Kolliker thought that there were groups of smooth muscle fibers arranged in radial bundles which did not form a continuous membrane but lay in the intervals between the blood vessels. Another group emphasized the muscular lamina situated in the posterior surface of the iris. This posterior membrane is described today in textbooks as the dilator muscle of the pupil.

The question arises as to what reflex stimuli produce dilatation of the pupil through the sympathetic pathway. No one has ever contended, apparently, that the dilatation of the pupil in dim light is dependent upon contraction of a dilator muscle activated along the cervical sympathetic pathway. However, many investigators have felt that the dilatation of the pupil in response to pain was mediated by the sympathetic fibers to the pupil. Bechterew in 1883 did not accept this theory and concluded that dilatation of the pupil resulting from painful stimulation is caused solely by inhibition of the activity of the third nerve (parasympathetic).

Anderson in 1904 noticed that reflex dilatation of the pupil occurred after removal of both superior cervical ganglia (sympathetic). However, after section of the parasympathetic fibers he found that dilatation of the pupil may be readily elicited by tactile and painful stimuli, but that the reflex dilatation ceases when

the cervical sympathetic trunk is also cut. He stated that the dilatation in response to pain is immediate with a second delayed response. The latter he thought was due to the action of adrenalin. He stated that the quick response was lost when the cervical sympathetic trunk was cut, but the delayed response remained. Only the quick response was obtained by taking a cat's forepaw in the hand or when the ear or hair of the neck was stroked. This was the type of stimulus producing a pure response directly through the cervical sympathetic trunk, according to Anderson.

The more modern studies have not all tended to confirm Anderson's conclusions. Gullberg, Olmsted and Wagman (1938) studied movements of the pupil using infrared photography. They felt that two forces caused dilatation of the pupil: first, active contraction of the dilator muscle; two, passive stretch of elastic tissues which tend to restore the resting configuration. Both of these forces act in the normal eye, but after the sympathetic fibers have been cut, only elasticity can function. The rate of dilatation is faster in the normal eye than when the sympathetic fibers have been cut. They concluded that the dilator muscle must begin to contract at the very start of the pupillary dilatation. After sympathectomy, the dilatation showed the slow straight curve of an elastic reaction. In the normal animal, the first part of the curve was steeper, demonstrating the action of the dilator muscle.

Ury and Gellhorn (1939) believed that dilatation of the pupil in response to emotional stimuli is effective through inhibition of the third nerve, since this form of stimulation evokes immediate dilatation in the sympathectimized pupil, but fails to do so when the oculomotor nerve is cut.

In 1940, Ury and Oldberg stated that reflex dilatation of the pupil in response to pain is mediated solely by central inhibition of the parasympathetic innervation. They pointed out that each iris is innervated by both homolateral and contralateral portions of the oculomotor nucleus (Crouch, 1936). It is therefore under the control of both crossed and uncrossed fibers. This explains the mechanism by which the pupils react as a unit to constrictor influences in the consensual light reflex.

They found that none of the responses of the pupil to afferent stimuli is lost when the sympathetic component is cut, but the magnitude of the reaction is diminished. This difference is due to the normal tonic action of the sympathetic fibers and not to their reflex excitation.

Ury and Oldberg summed up in a diagram the forces acting upon the iris to produce pupillary changes. They mentioned three types of stimuli, tending to constriction of the pupil. There is a cortical control, mediated, as far as is known, from the occipital lobe of the brain. There is also a pretectile influence, tending to produce constriction of the pupil. Finally, the brain stem mediates a constrictor tonus to the pupil independent of any light stimulus; thus during sleep the pupils are constricted.

The authors mentioned two types of influences tending to produce dilatation of the pupil. First, there is a dilatation dependent upon excitation of the sympathetic fibers to the iris. This is an invariant stimulus and is not affected by

any reflex changes. Secondly, the pupil tends to dilate due to reflex inhibition of the sphincter muscle and this effect is mediated through the parasympathetic fibers which run in the oculomotor nerves.

Bain, Irving and McSwiney (1935) used pupillary dilatation as an index for determining the presence of pain fibers in the splanchnic nerves. They found that stimulation of the central end of the splanchnic nerves produced a dilatation of the pupil. From their experiments, they came to the conclusion that this dilatation depended upon a pathway through the third nerve nucleus (parasympathetic). They found that after section of the third nerve on one side, the reflex dilatation of the corresponding pupil was abolished, but the reactions of the opposite pupil were unaltered. Even after the pupil with the third nerve sectioned had been constricted by instilling eserine solution, no response was obtained on stimulation of the splanchnic nerves. Eserine did not alter the sensitivity of the reaction on stimulation of the cervical sympathetic fibers. Transection of the spinal cord in the cervical region rendered both pupils unresponsive to splanchnic stimulation, although stimulation of any nerve entering the cord above the level of the spinal section evoked an immediate dilatation. Finally, section of the cervical sympathetic nerves was found not to alter either the rate or the degree of the reaction to stimulation of somatic or visceral afferent fibers. They concluded, therefore, that under the conditions of their experiments, the reflex pupillary dilatation was due, mainly if not solely, to inhibition of the third cranial nerve nucleus (parasympathetic) and not to impulses conducted to the dilator muscle by the cervical sympathetic nerves.

Seybold and Moore (1940) studied the relations of the parasympathetic innervation to reflex dilatation of the pupil of the cat. They sectioned the oculomotor nerve (parasympathetic) intracranially and immediately the pupil upon that side became dilated until the transverse diameter became 13 millimeters. In some of these animals the sympathetic pathway to the iris was interrupted at a later date, either by sectioning the sympathetic trunk or by removing the superior cervical ganglion. After this second operation, the pupils became narrowed to a new permanent diameter which varied within a range from 7 to 10 millimeters.

In order to study reflex dilatation after section of the parasympathetic fibers, it was necessary artificially to constrict the pupil by the addition of some miotic such as eserine. This drug constricted both pupils, but the pupil on the side of the third nerve section remained 1 or 2 millimeters larger than the pupil of the normal eye. When the full eserine effect was obtained the pupil on the operated side measured 2.5 millimeters whereas on the normal side the pupil measured only 1.5 millimeters. If the sympathetic pathway had also been interrupted, the pupil was smaller on the side of the sympathetic operation. Although the degree of constriction in response to eserine was decreased by parasympathectomy, the duration of the miotic effect was prolonged. Even after twenty-four hours when the normal pupil had regained full activity, the parasympathectimized pupil still showed a decided constriction.

The authors (Seybold and Moore) then attempted to evaluate the role of the

sympathetic and parasympathetic fibers in pupillodilation. The responses were studied to the following three stimuli; light adaption (that is the withdrawal of the normal light stimulus), painful stimulation (such as electrical stimulation of the perianal skin, or strong faradic stimulation applied directly to the sciatic nerve exposed under light ether anesthesia), or emotional excitement aroused by restraining the animal upon its back or exposing it to the barking of a dog.

During the stages of partial effect of eserine, the pupil of the normal eye dilated instantly in response to each of the stimuli listed. There was not the slightest visible dilatation of the pupil on the side of the third nerve section. From these experiments, it is apparent that the activity of the parasympathetic fibers constitutes the principal factor in the reflex pupillodilatation, elicited by withdrawal of light, painful stimulation and emotional excitement.

The sympathectomized pupil dilated readily on withdrawal of light. Under nembutal anesthesia no difference in the size of the two pupils was observed. In the unanesthetized state, however, it was noticed that while the response was prompt, the dilatation was not as great on the side of the operation. The sympathectomized pupil dilated readily in response to faradic stimulation of the sciatic nerve. The maximum diameter obtained by the pupil on the side of the operation was never as great as that of the opposite pupil, but the response was equally as prompt. After the sympathetic fibers were cut, the pupil underwent immediate dilatation in response to emotional excitement. Again, however, the dilatation was less than in the normal eye. One exception was noticed in animals in which the superior cervical ganglion was removed. There, prolonged stages of emotional excitement led to augmentation of the dilatation. This was considered to be an adrenalin affect.

The authors concluded that under ordinary conditions sympathetic dilator tone is remarkably constant, while parasympathetic responses are subject to extreme reflex modification. Consequently, changes in the size of the pupil depend upon reflex variation in the activity of the oculomotor nerve (parasympathetic). Section of the sympathetic fibers effects a diminution in the extent of the reflex dilatation.

The control of pupillary size was described by Langworthy and Tauber (1937) in the following way: "It may be said that the dilator or sympathetic fibers furnish a general tonic background upon which phasic movements of constriction act only as long as the stimulus is applied." Experimental work has shown rather conclusively that reflex responses of the pupil are dependent upon changes in the sphincter muscle either in the direction of contraction or relaxation of these muscle fibers. It would appear, therefore, that the sphincter muscle of the pupil alone is responsive to reflex stimuli induced by light, pain, and emotion. The influences acting upon the iris through the sympathetic pathway give the pupil a permanent tonic background of dilatation, allowing the sphincter muscle to act more freely.

If these facts are true, little importance can be attributed to dilator muscle fibers, even assuming that they are present in the iris. It makes it possible to consider and evaluate again the function of the blood vessels in the iris in respect to the background of tonic dilatation.

In summary, two antagonistic muscles balanced against each other, innervated by two separate groups of autonomic nerve fibers are not required to explain the pupillary movements. Instead, as in a case of striated muscle, the contraction of the sphincter muscle can be increased or decreased to produce the desired effect. If there is any dilator muscle present in the iris, it has little, if any role in the normal phasic activity producing changes in the size of the pupil. The iris undoubtedly has a property of elasticity acting against the sphincter muscle permitting the pupil to dilate promptly with relaxation of the sphincter.

ANATOMICAL CONSIDERATIONS

The original observations in this paper are largely morphological. This makes it necessary to introduce a general description of the iris structure (Magitot, 1921; Troncosa and Castroviejo, 1936).

The Iris

The retina of the eye has two protective mechanisms which guard it from excess of light; the retinal pigment and the iris. The iris may be considered as the diaphragm of a black chamber which forms the eye.

In order to provide for the perfect formation of images on the retina, it is necessary that both spherical and chromatic aberration should be overcome. This is accomplished largely by contraction of the pupil. The pupil is the central opening in the iris through which light passes to the retina; it acts in a manner similar to the stops in a camera. In order to obtain a clear image in a dull light, the pupil opens widely; conversely, if the light is too abundant, the pupil becomes small.

The iris can regulate the diameter of the pupil to control the rays of light necessary for sensory impression. This action is dependent on photomotor reflexes. It has a second role in accommodation and convergence, admitting the rays of light optimum for the curvature of the lens. This is controlled by the accommodation-convergence reflex. The iris also responds to other sensory and emotional impressions. It reacts strongly to pain. Responses of the iris are said to be greater in younger people than in older.

The iris has the form of a truncated cone resting on the lens. It is formed embryologically by the fusion of three layers, two from the optic vesicle and one from the uveal mesoderm. From a comparative anatomical point of view, the iris first was formed from the edges of the developing retina and the connective tissue element was added later. In some reptiles and birds (Geberg, 1884) the iris contains striated muscle; the smooth muscle is unusually well developed in marine mammals such as the whale.

From an embryological viewpoint, the outer mass of the iris is made up of two layers of mesoderm (Duke-Elder, 1932). The anterior one is originally stretched completely across the pupil as the pupillary membrane. As development proceeds, this portion gradually atrophies leaving traces behind. The final pattern depends upon the amount of atrophy which occurs. Localized patches of atrophy give rise to the formation of crypts. The pattern of these crypts, the blood vessels and pigment cells is characteristic of the individual and may be

used for identification. After the complete development of the iris, the lateral portion consists of two mesodermal layers, while the mesial portion consists of only one. The lateral portion makes up the area of the ciliary bodies. The line of junction between the two-layered and one-layered mesodermal portions of the iris is called the iris frill or circulus iridis minor. The lateral part will be called the ciliary portion and the mesial the pupillary portion.

The iris has a free and adherent border. The adherent border is inaccessible to view. The iris is three- to six-tenths of a millimeter in thickness. The thickness is least at the point of attachment. In looking at the mesial or pupillary portion of the iris, it appears serrated, due to the radial blood vessels. These form external and internal rows and leave a smooth circle around the pupil. In the iris there are two layers of pigment, one lying in the posterior epithelium and the other in the stroma. In the newborn the stromal pigment is absent, so that the eyes have a blue color. In the albino, there is no pigment in either layer. The difference in tint from blue to other shades is due to the pigment in the stroma.

On the surface of the anterior chamber, the stroma of the iris appears in transverse section to be in contact with the aqueous humor. However, this surface is covered with endothelium as can be observed with silver stains. This outer or mesodermal portion of the iris is the thickest. It is a vascular layer with chromatophores and other distinctive cells, covered anteriorly by endothelium and posteriorly by the two layers derived from the retinal epithelium. At the border of the pupil and also at the ciliary margin, there are deep depressions or stomata.

In the iris there are perivascular channels but no separate lymphatic system. In the stroma, there are other cells in addition to the chromatophores. Stromal cells are provided with finer and more delicate processes than the pigment cells. Wandering cells are found and granular cells from the blood. Another cell, heavily pigmented, and devoid of processes is found in the region of the ciliary margin in the sphincter muscle. It is probably derived from the pigment epithelium (Munch, 1907).

The inner portion of the iris consists of two layers, anterior and posterior, which correspond to the two layers of the primitive optic vesicle. The posterior is composed of cylindrical cuboid epithelial cells filled with pigment so that they can be studied only in the albino or after the pigment is removed. This pigment is made up of round grains and its function is to shut out the beams of light. The nuclei of these pigment cells are round and have a central position. This layer is homologous to the visual layer of the retina.

The more anterior epithelial layer gives rise to the sphincter muscle which surrounds the pupillary margin in the free edge of the iris. In order to observe this layer well, it is necessary to remove the pigment or study an albino animal. Lateral to the sphincter muscle, the layer is made up of a thin layer of cells which are thought by many to represent the dilator muscle of the iris. The muscle zone here is so thin that it scarcely exceeds 2 micra.

The sphincter muscle, medially, is arranged in thick, dense, parallel bundles

but tends to be thinner laterally. The peripheral border of the muscle blends into the fibers of the so-called dilator muscle. The so-called dilator is composed of cells which retain partially their epithelial character. Each cell is spindle shaped with an oval nucleus and is characterised by pigmented protoplasm. It trails off at one or both poles into a long, fiber-like process, staining like muscle tissue. These fiber processes run radially in the iris in a single sheet. The nuclei of the cells usually lie posteriorly, the protoplasm more anteriorly. Consequently, the so-called dilator appears on transverse section to be made up of two layers, a membranous layer anteriorly and a cellular layer posteriorly. At the ciliary margin of the iris, this layer is said to thicken considerably and send off prolongations into the stroma. Groups of cells run obliquely into the ciliary muscle and into the pectinate ligament, which provides a fixed attachment.

In certain fish, birds and mammals, an unusual mechanism is used to close the pupil (Magitot, 1921). An iris curtain falls over the pupil, to protect it from excess of light. This is known as an operculum and is made up of connective tissue and blood vessels. Apparently the operculum becomes turgescent under the influence of light. There is no muscle in the operculum and the movement appears to be duo purely to the dilatation or contraction of the blood vessels. This is a method of pupillary closure dependent entirely upon vascular changes and not upon contraction of smooth muscle.

The Chromatophores

The stroma of the iris is derived embryologically from the mesoderm. In the adults, it is made up largely of loose connective tissue, not grouped in bundles, but arranged around the blood vessels. Blood vessels and nerves pass through this layer and it contains tho pigment cells or chromatophores. The chromatophores are long, irregular shaped cells with branching processes (Lauber, 1908). They predominate upon the anterior surface of the stroma. In adult life they are filled with pigment which is more abundant in brunettes. The stroma also contains wandering cells and plasma cells derived from the blood.

At birth, the chromatophores in the stroma contain little pigment. The eyes of a newborn baby are blue due to pigmentation in the posterior layer of the iris derived from the inner cell layer of the optic cup. As pigment collects in the chromatophores, the color of the iris changes. A failure of normal innervation of the chromatophores in a sector or in the whole of the iris leads to abnormal pigmentation of this area; thus, normal people may show a segment of the iris which is blue in color whereas the rest of the iris and the iris of the other eye may have a different color. The iris may be of a different color in the two eyes (Horand, 1911).

The development of the chromatophores seems to be dependent upon the normal innervation of these cells. A lack of their pigmentation or heterochromia of the iris may result from an injury of the cervical sympathetic trunk and may be evident in cases showing a Horner's syndrome. Not only does paralysis of the sympathetic nerves lead to depigmentation of these cells, but the chromatophores have been observed to change their shape, lose their characteristic pro-

cesses and become spherical. The appearance of loss of pigment is dependent on the absence of the processes and the concentration of pigment granules in the spherical cell. After division of the ciliary vessels and nerves, the normal branching chromatophores in the choroid may be replaced by large round cells without processes.

These changes in chromatophores may be seen easily in lower forms and have been studied particularly in the frog. If the nerves to the iris of the frog are sectioned, the chromatophores at once contract and draw in their processes, (Krogh, 1927).

The nerves of the iris

There are three groups of nerves innervating the iris, having different functions and derivations: sympathetic, parasympathetic and sensory. The parasympathetic fibers reach the eye with the third nerve, the sympathetic fibers by way of the cervical sympathetic trunk and the sensory fibers from the trigeminal nerve. It is probable that most, if not all of the sensory fibers are derived from the trigeminal nerve and few run with the motor autonomic fibers.

The preganglionic sympathetic fibers of the iris are derived from the first and second thoracic spinal segments. About 1852, Claude Bernard became interested in the great cervical sympathetic trunk. Brown-Sequard recognized vasomotor nerves in this trunk. Budge (1852) showed that excitation of the cervical cord produced mydriasis. He believed the dilator fibers of the pupil had their origin in the fourth cervical segment of the cord. He thought of this as a paired and symmetrical area which was called the center of Budge. He found that stimulation of half the cord produced a unilateral response. Later, the origin of these fibers from the first and second thoracic segments was demonstrated. Later, François-Franck (1878) showed that the postganglionic dilator fibers of the pupil after leaving the superior cervical ganglion ran toward the ganglion of the trigeminal nerve and reached the orbit with the first branch of the fifth nerve.

Thus, the fibers of the first and second thoracic segments travel craniad in the cervical sympathetic trunk, to end around cells in the superior cervical ganglion. The fibers of the postganglionic cells are carried upward from here in the plexus around the carotid arteries until they reach the trigeminal nerve. They travel by the first branch of this nerve to reach the ciliary ganglion. They pass through or close to the ciliary ganglion without anastomosis to reach the eye. They leave the trigeminal nerve as the long ciliary nerves, two and sometimes three in number.

The parasympathetic fibers leave the central nervous system with the oculomotor nerve and end around cells in the ciliary ganglion. The postganglionic neurones are grouped at first as three or four short ciliary nerves, but they subdivide so that when they reach the globe there are about twenty. The exact number is variable. Short and long ciliary nerves, as well as the sensory fibers, penetrate the sclerotic coat of the eye around the optic nerve. They run through the choroid coat of the eye. The short ciliary nerves are parasympathetic and the long ciliary nerves are sympathetic fibers.

It has been suggested that each group of ciliary nerves supplies a definite sector of the iris. In general, however, the stimulation of any group causes a contraction or dilatation of the whole pupil. Langley and Anderson (1892) found that by stimulating individual groups of nerves in the choroid, they could produce either a restricted or a total effect upon the iris.

At their origin from the ciliary ganglion, the short ciliary nerves have a fine myelin sheath. In this connection, it should be remembered that the sphincter of the iris is composed of striated muscle in birds and some other forms. In the choroid of the eye, the short ciliary nerves branch and lose their myelin sheath.

Several investigators have described ganglion cells in the choroid and iris (Andogsky, 1897). It is often stated that there are isolated groups of two to twenty cells scattered along the nerves or included in the nerve trunk. The presence of these cells in the iris is a matter of some controversy.

The nerves enter the iris in large trunks which break up into a plexus containing both medullated and nonmedullated fibers (Dostoiewsky, 1886). These are considered to give off sensory fibers ending in the stroma, vasomotor fibers to the blood vessels and nerves to the muscle. The network of fibers is extraordinarily rich, so much so that it has been considered that every stroma cell and chromatophore receives its own nerve supply. The ciliary nerves run not only medially, but also radially in the iris and divide so that they form peripheral circular nerve plexuses composed of two or three circles (Meyer, 1878 and Kirpitschowa-Lentowitsch, 1911).

Agababow (1893 and 1912) gave a description of the nerves and nerve endings of the iris. In addition to the plexus formed by nerve trunks which has been described, he found upon the surface of the iris two peripheral plexuses: one superficial, and the other deep. These were very different in the dimensions of their mesh and in the size and varicosity of the fibers. In the deep layer the mesh followed the capillaries and took a position parallel to these vessels. There were triangular nuclei along the course of the nerves. The superficial layer did not follow the capillaries. The varicosity of these fibers was greater, the meshes were larger and it was possible to follow them almost to the pupillary margin. He thought that the superficial subendothelial plexus was sensory and the deep one vasomotor. In the region of the pupil, the myelinated fibers gave rise to thin varicose fibers which penetrated the terminal plexus. In this terminal plexus the isolated fibers ended, but it was not possible to learn how they terminated. The motor nerve fibers for the sphincter muscle ran parallel to the muscle fibers. The nerve fibers entered the muscle bundles, surrounded the cells and ended near the nuclei of the muscle cells. It was not possible to see a connection with the nucleus. The muscle cells were surrounded by numerous filaments which terminated at different points at the pole of the cell or in the region of the nucleus. There always existed a space between the nucleus and the ending.

Agababow found four types of nerve endings in the iris of the albino cat: (1) motor endings on smooth muscle, (2) vasomotor fibers in the walls of the blood vessels, (3) plate-like endings in the connective tissues which he thought subserved ordinary sensation, (4) endings which he considered proprioceptive since

cesses and become spherical. The appearance of loss of pigment is dependent on the absence of the processes and the concentration of pigment granules in the spherical cell. After division of the ciliary vessels and nerves, the normal branching chromatophores in the choroid may be replaced by large round cells without processes.

These changes in chromatophores may be seen easily in lower forms and have been studied particularly in the frog. If the nerves to the iris of the frog are sectioned, the chromatophores at once contract and draw in their processes, (Krogh, 1927).

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they were found in connective tissue between smooth muscle cells, where presumably they were affected by the contraction of the muscle.

Boeke (1932, '33 and '36) studied the nerve endings of the iris stained with silver. In these preparations, sections were made so that the continuity of structure was lost. He found nerve endings which looked like small rings or bellows lying among the connective tissue cells in the stroma of the iris. There was also a fine anastomosing net of neurofibrillar fibers running between and sending fine branches into the muscle fibers where they ended as intraprotoplasmic rings near the nucleus. He thought the first type of endings were sensory and the second, motor. He observed nerve fibers ending on blood vessels. He also found endings which he thought were sensory in the sphincter muscle and said they were not much more complicated than the motor endings. He was not certain that he saw ganglion cells in the iris. Boeke stated that the iris provided the best example of a plexiform innervation. He stated that in mammals a very complicated net-like plexus is found in the ciliary muscle. It has ring-like nerve endings inside the muscle cells and branches which anastomose everywhere with each other.

In this connection, it might be mentioned that Boeke (1940) has unique ideas concerning the innervation of autonomic structures. He has described an autonomic nerve plexus found in all the tissues of the body. The nerve fibers are closely related to the blood vessels, including the capillaries. He believed that they formed a terminal protoplasmic reticulum which may penetrate the substance of smooth muscle, fat or gland cells. Motor and sensory, sympathetic and parasympathetic fibers are thought to merge into this terminal net. There are also interstitial cells which are regarded as a true part of the plexus formation. From them arises the so-called intraprotoplasmic network by which nerve impulses are transmitted to various organs of response. Boeke thought that there was a protoplasmic continuity at the synaptic junction and no inert barrier was present between nerve fibers and the cells which they innervated. Terminal protoplasmic networks joined with the interstitial cells to form a syncytium.

Clark (1937) described the nerve endings in sections of the iris of the cat as stained by the pyridine silver method of Ranson. He pointed out that many of the nerve fibers of the trigeminal nerve did not pass through the ciliary ganglion but ran in small groups which fused with the ciliary nerves a short distance peripheral to the ganglion and close to the orbit. He observed endings upon the sphincter muscle which were usually, although not invariably, near the nucleus of the muscle fibers. He was not able to tell whether they were intraprotoplasmic or not. He claimed that after removal of the superior cervical sympathetic ganglion, the nerve endings upon the dilator muscle were lost, but he did not specify what he meant by the dilator muscle. He did not observe any ganglion cells in the iris of the cat.

Clark pointed out that the nerve fibers branch in great profusion among the smooth muscle cells of the sphincter. There are final ramifications running roughly parallel to the long axis of the muscle fibers. Where the nerves branch, there was usually a triangular expansion, showing their neurofibrillar structure.

In the course of the small subdivisions there were varicosities which varied in size and shape and at the endings of the fibers were seen small knobs or rings. There were certainly enough terminal fibers for the separate innervation of each muscle cell. He did not observe any endings in the cytoplasm of the smooth muscle cells. One fiber appeared to end around many muscle cells. There were quite similar endings in the connective tissue of the iris, near the pigment cells or the ciliary epithelium. Clark pointed out that it is difficult from histological examination to determine the function of nerve fibers. He thought that there was no plexus or reticulum. All the fibers and endings were discrete. He saw no ganglion cells in the iris.

Boeke (1932) also described the intraprotoplasmic position of end rings and end nets in the ciliary body. It seemed that every muscle cell received a nerve ending. He observed accompanying the blood vessels, a plexus of very fine, unmyelinated nerve fibers running in small bundles with intercallated nuclei. These bundles consisted of very delicate varicose nerve fibers which everywhere anastomosed with each other and formed a distinct network. He also found the nerves running so close to the endothelium layer of the blood vessels as to seem imbedded in it. Nerve fibers often lay in the same plane as the nuclei of the endothelial cells. Even the capillaries were encircled by nerve fibers. In addition to the vascular nerves, nerves were found running everywhere in the connective tissue of the stroma. They lay in loose anastomosing bundles and could be recognized by their extreme delicateness, their varicosity, and by the intercallated nuclei.

Boeke also found in the wall of the arteries of all sizes, an intricate network of delicate nerves. Beneath the superficial network in the adventitia there was found a second plexus which bordered on the media and arose from branches of the external superficial nerve plexus. This was characterized by a tremendous number of very fine nervous fibrils which crossed and recrossed each other in an indiscriminate manner and showed numerous loops, bands and small varicose swellings.

FACTORS INFLUENCING THE SIZE AND SHAPE OF THE PUPIL

The diameter of the pupil is essentially unstable. Its size during the day is influenced by all sensory impressions and emotional reactions of the individual. During sleep the pupil is small. The pupils react not only to light and accommodation but also to muscular effort and painful stimuli. Gripping of the hands or violent muscular effort cause dilatation of the pupil. Mydriasis is produced by vomiting. If painful stimulation is applied when the pupils are well illuminated, they can be seen to dilate. The pupil becomes smaller on any forceful attempt to close the eyelids. When the effort ceases, the pupil dilates. The response is bilateral (Behr, 1913).

Reflex pupillary responses begin to develop before birth. Newborn cats with their eyes still closed have pupils reactive to light. In man, the pupillary reflexes appear in the fifth fetal month and become active in the sixth. They appear first as a vermicular contraction of the iris. This is at first an energetic

contraction followed by slight dilatation and then a series of small oscillations. The latent period is very short. The human iris does not react to pain stimuli until three or four months after birth. The consensual response does not appear in those animals in which all the optic fibers cross. It is only where there are uncrossed fibers that both eyes respond to light stimulation applied to one. The response to convergence is slower than that to light. The pupil is inactive in extreme youth and in old age. The relative immobility of the pupil in old persons is due in part to senesence of the tissues of the iris which have lost their elasticity. The pupils may be said in general to be small in infancy, large during adolesence, moderate during middle life and contracted in old age.

The width of the pupils is controlled by a number of factors, such as light, accommodation, mental state and age. Any given width of the pupil is maintained by the equilibrium of muscle tension, tension of the walls of the blood vessels and the elasticity of the iris. If the cervical sympathetic supply to the iris is cut and the oculomotor nerve is paralysed with atropine, the diameter of the pupils in man is about 7 to 8 millimeters. This new diameter represents the point of elastic equilibrium. If the sympathetic influence alone is removed, all the normal reactions of the pupil are present but the excursion is limited to a maximal dilatation of 5 to 6 millimeters instead of a normal dilatation of 10 to 11 millimeters. The sphincter muscle alone, antagonized by the elasticity of the iris, is able to perform the normal movements of the pupil by contraction and relaxation. If the oculomotor nerve is cut or the endings of the parasympathetic fibers are paralysed by atropine, the pupil will dilate to 12 or 13 millimeters and maintain this position. The mechanism controlled by the cervical sympathetic fibers, whatever it may be, acts physiologically more or less like a spring, increasing the elastic equilibrium of the iris from 5 to 6 millimeters to 12 or 13 millimeters, thus allowing a wider range for contraction and relaxation of the sphincter.

Stimulation of almost all portions of the cerebral cortex with an electric current produces bilateral dilatation of the pupils. However, from localized areas of the occipital lobe constriction of the pupils can be elicited. It is probable that the dilatation produced on stimulation of other areas of the cortex is related to painful excitation.

Stimulation of the sciatic nerve produces dilatation of the pupils after a relatively short latent period, the reaction then receding rapidly. There is then a second dilatation which lasts longer and it is thought that the second response may be an adrenalin reaction. This latter mydriasis may be obtained after the cervical sympathetic trunk has been cut. Stimulation of any peripheral nerve elicits a similar pain response.

In sleep, the pupils are always contracted. When the lids are closed against resistance, the pupil can be seen to contract. This is called the orbicularis reflex and is supposed to be related to associated movements of the eyes upward. The oculo-sensory reflex is brought about by sudden sensory stimulation of the cornea or conjunctivae. Fear or emotional excitement cause a dilatation of the pupil. Stimulation of the cochlear or vestibular nerves produces changes in the pupil. Dilatation of the pupil follows extreme exhaustion of the central nervous

system. A blow on the eyeball may cause a paralytic dilatation of the pupil which may last for hours or days.

Section of the sympathetic fibers of the iris does not abolish the possibility of reflex dilatation of the pupil. The diameter is similar to that of the pupil during sleep. Convergence and light reflexes persist although decreased in amplitude. There is still dilatation of the eye in response to pain, fright, convulsions and asphyxia. The miosis produced by section of the sympathetic trunk is not lasting and does not persist in intensity until regeneration of the nerves takes place (Brucke, 1931). There is a certain recovery of tonus in the peripheral structures themselves which has also been demonstrated in the case of peripheral blood vessels. Removal of the cervical sympathetic ganglia does not change this finding so it may be assumed that it is not dependent upon impulses from the peripheral ganglia traveling along post-ganglionic fibers. Some weeks after the operation, the diameter of the pupil upon the side on which the superior cervical sympathetic ganglion has been removed may become larger that that upon the sound side. This is called the paradoxical pupil reaction. It is probably due to an oversensitivity of the muscle of the iris to adrenalin.

After removal of the ciliary ganglion, resulting in injury to the postganglionic parasympathetic fibers, the pupil shows maximum dilatation whereas after section of the third nerve containing the preganglionic parasympathetic fibers, the dilatation is not complete. Section of all the ciliary nerves, including the sympathetic and parasympathetic fibers, produces a complete paralysis of the iris muscle. Section of one short ciliary nerve produces a paralysis of a corresponding segment of the iris. This is not strictly true. There may be a slight deformation of the iris, but the corresponding segment is not completely paralysed. Sometimes on stimulation of one short ciliary nerve, a portion of the sphincter muscle contracts so that constriction of the pupil is predominantly on one side. Excitation of one long ciliary nerve may produce a dilatation of half of the iris.

In summary, the dilatation of the pupil after removal of the ciliary ganglion is maximal and all reflex activity is apparently abolished. The contraction of the pupil after removal of the superior cervical sympathetic ganglion is mild and all the reflex activity of the pupil is still present. Section of the third nerve will not produce a greater dilatation of the pupil than section of the optic nerve which suppresses the retinal reflexes.

DRUGS WHICH ACT UPON THE PUPIL

Adrenalin, ergotoxin, and cocaine influence the pupil through the sympathetic neuromuscular mechanism of the iris. The drugs which act on the parasympathetic neuromuscular function of the iris are atropine, pilocarpine, eserine, cholino and muscarine. An excellent description of the action of these drugs on the iris was given by Guyton (1940).

Atropine will exert its effect even after the death of the animal. When a weak solution is applied, the sphincter is paralysed before the ciliary muscle. The effect is most marked in young people. Inasmuch as the drug will act upon the nucleated eye, its effect must be directed primarily to the smooth muscle itself.

Eserine or physostigmine has a purely local effect, producing a spasm of the

sphincter muscle. The beginning of its action is of the nature of hippus, with each small contraction greater than the preceding one. The reaction of the pupil to light persists, if the dose is not too great. Following section of the third nerve, eserine has its usual action. After eserine has been applied, stimulation of the sympathetic fibers usually induces dilation of the pupils. When the sympathetic fibers to the pupil have been cut, the action of eserine makes the pupil smaller on the side of the section. Pilocarpine has an action on the iris less lasting than eserine. Muscarine produces contraction of the pupil, acting more on the ciliary muscle than on the sphincter.

The dilatation of the pupil caused by cocaine does not suppress pupillary reflexes. The dilatation is not absolutely regular and involves particularly the internal, inferior portion of the iris. Cocaine augments the activity of atropine. Adrenalin produces a dilatation of the pupil by stimulating the sympathetic mechanism.

Shen and Cannon (1936) found that the injection into the conjunctival sac of acetylcholine (1 to 5 per cent) had no effect on the normal iris. Direct application of acetylcholine to the isolated pupillary sphincter caused a prompt maximal contraction of the muscle. After excision of the ciliary ganglion or section of the short ciliary nerves instillation of concentrated acetylcholine made the paralysed sphincter contract.

Dilute acetylcholine (1/10 to 1/1000 per cent) had no effect on such a sensitized sphincter, but it did produce a marked contraction if eserine had been instilled previously. Such a powerful miotic action of the drug cannot be due to the effect of eserine alone. Sensitization of the pupillary sphincter muscle in the cat as a result of denervation has only a brief latent period. Hypersensitivity to acetylcholine is found even in an acutely denervated sphincter. Maximal sensitization is attained within 24 hours after the excision of the ciliary ganglion.

Heath and Geiter (1939) and Heath and Sach (1940) endeavored to study the so-called dilator muscle by examining the response to drugs of isolated radial strips of the iris after the sphincter muscle had been removed. They used albino rabbits in these experiments. They found that these isolated radial strips of the iris were caused to contract by epinephrine and neosynephrin in physiological doses. Application of atropine relaxed the contracted segments. The effect was dependent upon the relative concentration of the antagonistic drugs.

Part II. Innervation of the Iris of the Albino Rabbit as Related to Its Function

Method

The albino rabbit was used in carrying out anatomical studies of the iris. This animal is particularly useful for the purpose, in that the iris is free of pigment, which is an obstruction to histological studies. Undoubtedly, there are minor variations in the structure of the iris in different mammals; certainly in different vertebrates, the structure of the iris undergoes important modification.

The objection is often raised to comparative anatomical experiments of this type that the findings in one animal do not apply to others, inasmuch as the structure and innervation vary widely in different forms. We do not believe this objection is valid; it is probable that the general principles of innervation are similar in all mammalian forms. In the studies of the urinary bladder, it was found that the anatomical findings observed in the cat bladder apply with minor variations to the bladder of other mammals and of man.

Cross sections of the iris were made and stained with hematoxylin-eosin and connective tissue stains. These were used as control preparations, giving a general orientation of the structures to be found in the different layers of the wall of the iris. In all of the other studies, the iris was examined as a total mount, flattened out upon the slide. It was found that in the fresh and also in the hardened orbit, it was possible to uncover the iris and easily tear it away from the connections with the choroid membrane upon its outer edge. The iris then could be flattened under a cover slip and studied with an oil-immersion lens of long working distance as well as with all the lower powers of the microscope.

In one portion of this study, we were interested in learning the arrangement of the blood vessels in the iris, especially those forming the ciliary vessels. Accordingly, a dilute solution of India ink was injected through the heart into a recently killed animal. The injection was made at a pressure approximating the systolic pressure of the blood and was continued until the injection of the vascular system of the iris seemed reasonably complete. The orbits were then removed and fixed in formalin. After fixation was complete, the iris was removed, dehydrated, cleared and placed flat upon the slide.

In most cases, the iris was stained vitally with a dilute solution of methylene blue. The dye was injected through the heart, immediately after the death of the animal. The method of vital staining as used in this laboratory need not be described here in detail, inasmuch as it has been considered in a number of previous papers (F. Kleyntjens and O. R. Langworthy, 1937; O. R. Langworthy and E. L. Murphy, 1939; and O. R. Langworthy and F. H. Hesser, 1940). It is enough to point out the modifications in the staining method that were used in dealing with the iris. In the first place, it might be mentioned that we have found that American preparations of methylene blue which are suitable for staining blood cells do quite well for vital staining of neurones and we no longer rely on the methylene blue (nach Ehrlich) as supplied by the Gruebler Company in Germany.

The injection was continued until the tissues of the iris became completely saturated with the dye. It will be remembered that when this dye is introduced into the body, it is rendered colorless, due to the lack of oxygen. The dye has an affinity for neurones and when oxygen is supplied again, these structures become blue due to oxidation of the dye in them. It became necessary to find some method for introducing air in contact with the iris to produce the oxidation. Accordingly, after the iris appeared to be well diffused with the dye, the needle of a hypodermic syringe was introduced into the anterior chamber of the eye and air was pumped into this cavity. This process was continued until the iris

turned a uniform blue color. When the oxidation seemed to be complete, the iris was exposed and quickly removed. It was then placed in cold ammonium molybdate solution in the ice box over night. The preparation of this solution has already been described in previous papers.

On the following morning, the iris was placed in two changes of glycerin for a half hour each. It was transferred through two changes of 95 per cent alcohol for five minutes each and two changes of absolute alcohol for five minutes each. The iris was afterward placed in cedar oil for three hours and mounted in balsam upon a slide.

These preparations kept the stain well and many of them have remained in good condition for over a year. The use of cedar oil is an important new modification of the technique. Methods were devised by which it was possible to intensify the staining of the nerves, if the color began to fade. The cover slip was removed and further balsam placed over the iris without renewing the cover slip. Preparations without a cover slip appeared to absorb enough oxygen from the air so that the color of the nerves was intensified. These preparations lent themselves to study quite as well as those with a cover slip. Also, it was often useful to turn the preparation over upon the slide so that the deeper layers were brought into contact with the microscope lens and could be seen more clearly. In this case, the preparations were placed in xylol again until the balsam was removed and then remounted with the sides reversed. This type of treatment appeared to do the preparation no harm.

By changing the focus, it was possible to bring into view the different layers in the wall of the iris, giving a three dimensional picture. It was important, therefore, to understand from the beginning the structure of the entire iris wall in order to identify the layer which was in focus. In the preparations with the blood vessels injected, it was possible to follow the vascular pattern throughout its entire course in the iris wall and see the arrangment in the different layers of the iris. In the case of the nerves, they could be followed from the point where they entered the outer edge of the iris to endings upon the sphincter muscle. The nerves and nerve endings were studied under all powers of the microscope including the oil immersion.

From the standpoint of vital staining, the iris was even more instructive than the bladder. It was possible to mount only small portions of the total wall of the bladder of the cat upon slides, but the entire iris could be viewed as a whole. This method of spacial microscopy is of extraordinary value in making it possible to follow the continuity of structure in thin membranes and the iris is an ideal place for this type of anatomical study.

It should be mentioned that no two vitally stained preparations are exactly similar in the method in which the tissues react to the dye. Often structures other than nerves stain well, and this aids in orientation of the material. In the bladder, nerve cells were often stained. No nerve cells have been seen in the iris of the rabbit, although they have been described by other workers. It is possible that the pigment cells were confused with nerve cells. Also, the fat cells stained well in the urinary bladder. There are no fat cells in the iris of the albino rabbit.

Elastic fibrous tissue cells stained in the iris. These are relatively rare and occur in the substance of the sphincter muscle close to the free pupillary margin. The pigment cells scattered in the collagenous connective tissue of the outer layers of the iris often were differentiated and their processes could be seen clearly. These will be illustrated in photographs. The smooth muscle in the wall of the arteries also took the stain in many instances. In the case of the veins the endothelial cells of the walls were blue. It was easy in the vital preparations to distinguish arteries from veins.

EFFECT OF CHANGES IN BLOOD PRESSURE UPON THE SIZE AND SHAPE OF THE PUPIL

As has already been mentioned, earlier anatomists and physiologists believed that changes in the size of the pupils were dependent upon constriction and dilatation of the blood vessels of the iris. Even when a specific sphincter muscle was found, the dilatation of the pupil was ascribed largely to vascular influences. Thus it was thought that a constriction of the blood vessels would make the pupil dilate and dilation of the blood vessels would cause the pupil to contract. If the recent physiological experiments which have been mentioned here are reexamined from a critical point of view, it may be observed that many of the phenomena ascribed to the sympathetic nerve muscle action could be explained by changes in the caliber of the blood vessels or to contraction of the smooth muscle in their walls.

Gellhorn, Darrow and Yesinick (1940) observed that an increase in blood pressure produced by adrenalin or infusion of Ringer's solution led to a decrease in pupillary diameter. A fall in blood pressure induced by amyl nitrite led to a dilation of the normal and sympathectomized pupil.

Our own attempts to inject the blood vessels of the iris with India ink and methylene blue gave results which bear upon this whole question. In making these injections we endeavored to keep the pressure of the fluid which was introduced through the heart at approximately that of the normal blood pressure of the animal. In order to make the injection complete, however, it was often necessary to raise the pressure somewhat or to cut down the outflow of the fluid through the venous channels, causing the vascular system to be distended with fluid. Under these conditions the size of the pupil could be altered at will by changing the pressure of the fluid entering the vascular system. When the pressure of flow into the heart was increased, the pupils became smaller, whereas if the pressure was decreased, the pupils immediately enlarged in size.

In several of the animals injected with India ink, the superior cervical ganglion had been removed on one side several days previously. The pupil upon that side was smaller than the other. It was hoped that this difference in size of the pupil could be maintained and possible differences in the caliber of the blood vessels examined. When the pressure of flow of the India ink was made sufficient to cause a complete injection of the blood vessels, both pupils became small and usually of equal size. It appeared, therefore, from this artificial injection method that the pressure of fluid in the blood vessels of the iris has a significant effect upon the size of the pupil. If a good injection with the India ink was obtained,

the pressure had to be high enough so that in most cases both pupils were equally small.

Moreover, it was observed that the shape of the pupil was often altered during the period of injection. After the India ink had been injected into the arterial system, portions or sectors of the iris often stained first, leaving other areas uninjected with the ink. A small sector or half of the iris might stain well. Under these conditions, the iris became wider in the areas where the blood vessels had filled with ink. This caused the pupillary border of the iris to flatten upon the side where the blood vessels were injected with ink, producing irregularity in the shape of the pupillary margin. As the injection went on and all portions of the iris became injected with ink, the pupil again became fairly round, although some irregularity in the shape of the pupil might remain. From these results, it may be assumed that dilatation of the blood vessels in sectors of the iris causes a greater expansion of the iris in these regions and a flattening of the pupil margin in the area of the injection. Irregularity of the pupils, therefore, may be related to variations in the dilatation of the blood vessels in different sectors of the iris.

In summary, the injection experiments demonstrated that the size of the pupil could be varied by changing the pressure of fluid that was injected in the blood vessels. It is probably fair to assume that in normal individuals the pupillary size is influenced to some extent at least by the circulation through the iris. The shape of the pupil may be altered by unequal injection of different portions of the iris. In the area where the vessels have been injected with ink, the iris expands and the pupillary margin becomes flat, whereas the iris is narrower and circular in outline in portions not injected with the ink.

STRUCTURAL ORIENTATION BY MEANS OF CROSS SECTIONS OF THE IRIS

The preparations which form the real basis of the experimental study are total mounts of the iris either with the blood vessels injected with India ink or the nerves stained with methylene blue. In order to interpret the findings in these preparations, it is necessary first to obtain an orientation as to the structures composing the iris wall. For this purpose, cross sections were cut, and stained with hematoxylin and eosin or with connective tissue stain. Photographs from this material are shown in figures 1 to 5.

It is helpful to realize that the iris is derived embryologically from three distinct layers. The two inner ones originate from the invaginated optic cup. In the retina itself, the inner of these layers forms the nervous tissue and the outer, the pigmented choroid coat. The anterior portion of the optic cup is differentiated to form two layers of the iris. To this is added an outer or third layer from the mesenchyme. These three layers are not present in all vertebrate forms, but may be seen clearly in mammals.

It is not feasible to demonstrate the structure of the entire width of the iris wall in one photograph. Figures 1 and 2 show the free inner margin of the iris next to the pupil, including the sphincter muscle. Figures 3 and 4 illustrate the more peripheral portions of the iris, including the area of the so-called dilator muscle and the arrangement of blood vessels and connective tissue. The blood

vessels of the ciliary body are seen clearly in figure 3. Figure 4 is a higher power of an area similar to that shown in figure 3 and figure 5 is a still higher magnification, illustrating the arrangement of the collagenous connective tissue and pigment cells or chromatophores in the wall of the iris.

The inner layer of the iris wall representing the inner layer of the primitive optic cup is made up of a single layer of cylindrical cells with round central nuclei. In most animals, these cells are filled with pigment, so that they cannot be seen clearly. They can be made out fairly well in the illustrations, particularly in figures 4 and 5. There they form the uppermost layer of cells.

The sphincter muscle of the iris is derived from the outer layer of the primitive optic cup and is shown in figures 1 and 2. The muscle surrounds the pupillary margin of the iris. In the most mesial free margin of the iris, it forms a large mass of circular muscular tissue. It then continues laterally in an attenuated form as can be seen in the figures. The more lateral muscle fibers have a less circular arrangement and tend to spread out longitudinally toward the attached edge of the iris. As can be seen in figure 2, muscle fibers continue in the thin layer for a long distance laterally, blending with the cells which are supposed to make up the dilator muscle. There is then no well defined lateral edge of the sphincter muscle. The entire outer layer of the primitive optic cup is differentiated into cells having something of the appearance of smooth muscle. Laterally toward the attached surface of the iris they are few in number and tend to occur in small bundles. Here they are known as dilator muscle. More mesially, these fibers are not well delimited from the sphincter muscle.

The cells of the so-called dilator muscle have some of the characteristics of smooth muscle. They can be seen in figures 3 and 4 and it will be noticed that in certain places they form bundles extending outward into the stroma of the iris. They are near the upper portion of the iris in figure 4 and to the right in figure 3. In general the nuclei of these cells tend to lie internal to the cytoplasm. The cytoplasm may show poorly defined striations only at the two ends of the cells or throughout almost all portions of the cytoplasm external to the nucleus which is displaced medially.

The external wide layer of the iris is made up largely of collagenous connective tissue surrounding blood vessels and branched pigmented cells or chromatophores. This layer is thin at the free edge of the iris and becomes wider in passing toward the attached edge. In the photographs many of the blood vessels are filled with India ink. The blood vessels are small near the medial portion of the iris as seen in figures 1 and 2. Many scattered pigmented cells may be seen in this layer. The arrangement of the fibrous tissue is shown in figure 4 and under higher magnification in figure 5. In following the layer laterally, it will be observed that the blood vessels are larger than at the free edge of the iris.

In summary, the iris is derived embryologically from the two layers of the primitive optic cup with a superimposed layer of mesenchyme. The inner layer of the optic cup gives rise to a single layer of cylindrical cells which are filled with pigment in most forms. The well developed sphincter muscle and the cells which are supposed to make up the thin dilator muscle are derived from the outer

layer of the optic cup. The outer, mesenchymal layer consists of collagenous connective tissue fibers forming a loose network supporting blood vessels and branched pigment cells or chromatophores. The albino rabbit has the advantage for experimental work that the iris pigment is lacking. The sphincter muscle of the iris is well defined at the pupillary edge of the iris. Laterally, it has no distinct edge. Here the fibers tend to lie in a radial direction, to be few in number and arranged in small groups. These can be followed toward the attached edge of the iris and are described as the dilator muscle.

A STUDY OF THE BLOOD VESSELS OF THE IRIS INJECTED WITH INDIA INK

The arrangement of the blood vessels is shown well in the preparations of the iris in which the vessels have been injected with India ink (figs. 6 to 24). For the purpose of orientation, the arangement of the blood vessels in a portion of the iris is demonstrated in figure 6. The pupillary margin lies at the right edge of the picture. At the left edge, the continuity between the thin choroid coat of the eye and the iris may be seen. The circular ciliary vessels appear as black bands extending around the iris. The groups of vessels forming the ciliary bodies extend downward toward the free margin of the iris and also outward toward the attached edge. There are two distinct groups of vessels which supply the ciliary bodies. Many short ciliary vessels penetrate the iris from the choroid coat and extend into each of the ciliary processes at their lateral extension. Later we will show their structure more clearly in preparations where the long ciliary circulation was not injected. There are two long ciliary vessels entering opposite poles of the iris. The position of one of these vessels can be seen in the left lower edge of the picture. These enter peripherally and then turn to form a circular vessel extending entirely around the iris wall so that the arteries of the two sides anastomose at the points farthest from their origin. There are also long ciliary veins which drain the iris in addition to short ciliary veins which accompany the short ciliary arteries.

Two total mounts of the whole iris are shown in low power photographs in figures 7 and 8. These preparations are from the same rabbit. In the case of the iris illustrated in figure 8, the superior cervical sympathetic ganglion was removed 80 days before. It will be noticed at once, that the pupil is smaller in the iris where the sympathetic fibers were cut. Inasmuch as the ganglion was removed, there was no chance of regeneration of the fibers. The ciliary vessels have been injected in these two mounts of the iris, producing a fairly thorough injection of the total blood supply of the iris wall. It will be observed at once that the vessels are more dilated in the iris whose sympathetic innervation has been cut (fig. 8). This is seen particularly in the pupillary portion of the iris.

Figure 7 illustrates the neat way in which the iris can be separated from the adjacent structures, dehydrated, cleared, and preserved as a total mount. Some choroid clings to the lower edge of this preparation and is seen folded over at the edge. The long ciliary vessels enter the iris at the lower right and upper left margins, at about the point where there are folds in the preparation. Turning now to the ciliary bodies, it will be noticed that they extend inward, almost to the free pupillary margin, and that these ciliary processes are better developed at the

two points in the iris farthest from the place where the long ciliary arteries enter. The general arrangement of the blood vessels is clearly shown in figures 7 and 8. It should be mentioned that the same magnification was used in taking these two pictures and it will be noticed at once that the pupillary portion of the iris is wider in figure 8 than in figure 7. Also, it should be observed that there is a much greater injection of the ciliary processes extending down toward the free margin of the iris in figure 8 than in figure 7.

The next three figures (9, 10, 11) illustrate the prolongation of the ciliary processes extending medially toward the free margin of the iris. Even in a single preparation of the iris, these assume different forms as has already been pointed out in the last two photographs. Midway between the points where the two long ciliary arteries enter the iris, the medial prolongations of the ciliary bodies extend further downward toward the free edge of the iris, whereas close to the point where the long ciliary vessels enter, the ciliary bodies in their medial prolongation tend to be blunter. However, if we take one spot upon the iris and compare it in different preparations and also in the normal and after section of the sympathetic fibers, we find that the medial prolongations of the ciliary processes may assume different shapes at different times and under different circumstances. These variations are shown in the figures which have been mentioned (9, 10, and 11).

It will be noticed that the ciliary processes have a complicated structure, corresponding in some ways to that seen in the choroid plexus or in the glomeruli of the kidney. Thus, an artery runs downward in the ciliary process, giving rise to a number of small arterioles and capillaries which extend across the ciliary body and enter the ciliary vein. This will be illustrated further in the photographs which follow. In figure 9, the vessels of the medial expansion of the ciliary process appear to be contracted tightly together to form a ball shaped structure, which does not extend downward far toward the free margin of the iris. Exactly the opposite picture can be seen in figure 10. Here the vessels of the ciliary body are extremely loosely arranged and extend far downward toward the pupillary margin. These groups of vessels in the ciliary process give very much the appearance of coiled springs and it seems entirely possible that by contraction or dilatation of these vessels, or by contraction or relaxation of their muscular walls, the width of the iris and the diameter of the pupil may be modified.

Figure 11 gives a better picture of the contraction of the mesial portions of the ciliary bodies. In this case it will be noticed that the vessels appear to be very tightly pulled together, rather than lying expanded as in the last picture.

It will, of course, be realized that the arrangement of blood vessels is quite different in the various layers of the iris and this can be observed in the preparation by merely changing the focus. Thus, somewhat superficial to the ciliary processes which have just been described, there is a group of arteries of small size having the formation seen in figure 12. These are the arteries of the iris. It will be noticed that these blood vessels are also twisted and coiled, having a spring-like shape. They also extend mesially toward the edge of the pupil. It will be seen later that they are enveloped in connective tissue capsules and tend to cause folds on the surface of the iris which can be recognized in the eye of the living

prolongation extending toward the free margin of the iris and so far we have given little consideration to the lateral extension of these processes which are usually obscured by the circular vessels of the iris. The lateral prolongations of the ciliary processes are seen in figure 22, in the preparation where only the short ciliary vessels are injected. This shows the manner in which the large vessels break up into a plexus of smaller vessels making up the ciliary bodies.

Possibly one other observation in this study of the blood vessels of the iris deserves consideration. The injections were made at a relatively low pressure, scarcely more than that of the systolic blood pressure and in none of the preparations was there any rupture of the blood vessels, causing an escape of injection material into the tissues of the iris. However, in many of the preparations, we found circular dilations of certain small ciliary blood vessels, having the appearance of small aneurysms. These dilations are shown in figures 23 and 24. At a recent meeting of the American Association of Anatomists similar small sacular dilatations were described in the blood vessels of the posterior spinal ganglia. The significance of these findings is not understood, but it is likely that they are of the nature of an embryological defect.

In summary it has been demonstrated in these preparations injected with India ink that the medial projections of the ciliary processes extend down in the connective tissue toward the pupillary margin. The ciliary processes have somewhat the structure of coiled springs. Apparently the blood vessels are able to dilate and contract, changing to a considerable extent the contour of the ciliary bodies. In a preparation in which the sympathetic fibers to the pupil had been cut some days previously, the pupil was constricted and there was a greater dilatation of the blood vessels in the wall of the iris than in the opposite normal eye. We have previously seen that the size and shape of a pupil is influenced by the amount of dilatation or contraction of the blood vessels of the iris.

Other arteries of the iris, not connected directly with the ciliary bodies, also have a coiled appearance passing from the lateral edge of the structure toward the pupillary margin. The plexus of small arteries and capillaries in the wall of the iris and at the pupillary margin has been demonstrated in these preparations.

STRUCTURES OTHER THAN NERVOUS STAINED IN THE IRIS OF THE RABBIT BY THE VITAL METHYLENE BLUE TECHNIQUE

It has already been mentioned that the structures stained in the iris with methylene blue vary somewhat with each individual preparation. Although a great deal of uniformity obtains, groups of cells other than nervous tissue are often rendered visible and when stained along with the neurones help greatly in the interpretation of the findings. This is especially important since it is not possible to apply satisfactory counterstains to these preparations.

There are a few elastic fibers in the iris which usually lie close to the free margin of the pupil in the substance of the sphincter muscle. One fiber of this type with its nucleus is shown under high magnification in figure 25. The free margin of the iris is seen on the upper edge of the picture and along the edge of the iris lies the protoplasm of another elastic fiber. A third elastic fiber with its characteristic twisted nucleus is demonstrated in figure 26.

The chromatophores scattered in the connective tissue are somewhat more numerous laterally in the ciliary portion of the iris than near the free margin. They may be seen under low magnification in figure 27. Their relationship to nerves and nerve endings will be discussed in detail later. A high power photograph of one of these pigment cells is shown in figure 28. It will be noticed that the cell gives rise to complicated branching processes which give it something of the appearance of a nerve cell. It is probable that chromatophores have often been mistaken for nerve cells. Later, we will see that they are very closely associated with nervous structures in the iris.

In many cases, the smooth muscle of the arterial walls is stained well with methylene blue giving a good orientation as to the position and arrangement of the vessels in relation to other structures. Sometimes, the nerve endings on the muscle are stained at the same time; the innervation of the vessels will be described in a later chapter. In figure 29, small arteries can be observed in the iris wall. These are running outward toward the free margin. It will be noticed that the smooth muscle is not distributed evenly along the arterial wall, but is most concentrated at the point where the vessel curves or twists. In previous pictures, it has been shown that these arteries of the iris tend to pursue a corkscrew course like coiled springs. It is probable that the smooth muscle in the wall of the vessels can, by contraction and relaxation, control the coiling or straightening of the arterial wall and influence the width of the iris. This uneven distribution of the muscle in the walls of small arteries is seen even more clearly in figure 30. Figure 31 shows a somewhat larger artery with a thicker layer of smooth muscle in its wall.

The veins can be recognized in these preparations inasmuch as they take the stain somewhat differently. There is no smooth muscle evident in their walls, but the endothelial lining of the vein stains in many instances. Thus, with the proper staining, it is possible immediately to tell an artery from a vein. In figure 32, a small vein can be seen, with the endothelial wall stained. Finally in certain cases, the capillaries stain, so that one can see the nuclei of the endothelial cells and a faint outline of the wall of the capillary. The appearance of the capillaries with the methylene blue stain is demonstrated in figure 33.

In conclusion it has been shown that orientation in the preparations of the iris stained with methylene blue is favored by the fact that structures other than nerves often stain with the dye; thus we have been able to demonstrate fibrous connective tissue cells, chromatophores, smooth muscle in the wall of the arteries, and the endothelial lining of veins and of capillaries. When the chromatophores, for example, are stained, it is often possible to see the relationship of terminal nerve fibers and endings to them.

A GENERAL SURVEY OF THE NERVES AND NERVE ENDINGS IN THE IRIS OF THE ALBINO RABBIT

The nerves enter the peripheral edge of the iris from the choroid layer of the retina. They are of great abundance and tend to form two or three rows of anastomosing loops extending circularly around the iris (fig. 34). When these

nerve trunks are examined with the higher powers of the microscope as illustrated in figure 35, they are found to be made up of large myelinated fibers and groups of small unmyelinated fibers. Thus, the trunk shown in the picture contains one or two heavily myelinated fibers and numbers of small fibers. The small fibers when seen in the vital preparations do not stain as a continuous thread, but rather as a series of dots. The myelinated fibers tend to branch profusely and extend centrally to the margin of the pupil. They apparently end in all portions of the iris wall, but particularly around the pupillary margin. The general arrangement of these fibers is shown in figure 36 and again in figure 37. In both these pictures, but especially in 36, numbers of pigment cells may be observed. Figure 37 gives an idea of the manner in which the nerve fibers branch in the wall of the iris. Both photographs show a number of unmyelinated fibers running across the field. The heavy myelinated fibers, at points along their course, show peculiar varicosities; one of these is illustrated in figure 38. It occurs close to the nucleus of a cell which we take to be an endothelial cell in the wall of a blood vessel. The nature and function of this varicosity is not understood. In this same picture, the fine dots indicate an unmyelinated fiber.

Large groups of unmyelinated nerve fibers extend toward the margin of the iris and end finally as motor endings upon the sphincter muscle (figs. 39 and 40). These fibers extend mesially toward the sphincter muscle from its outer surface, then turn downward, become parallel to the muscle fibers, and run parallel to the muscle cells, giving off nerve endings. In figure 39, the pupillary margin of the iris is shown at the lower edge of the photograph. The position of the sphincter muscle is indicated inasmuch as the nerve endings which stain black run parallel across the field in the same direction as the muscle fibers. Lateral to the muscle can be seen a plexiform arrangement of fine nerve trunks. These are the parasympathetic fibers which extend downward to innervate the muscle. This same feature is illustrated again with greater magnification in figure 40. At the lower edge of the picture can be seen parallel smooth muscle fibers of the sphincter muscle. Although these fibers are not stained, they are somewhat outlined in the picture due to their differences in refraction. In the upper portion of the picture, groups of fine unmyelinated nerve fibers can be seen extending downward to innervate the smooth muscle fibers. They dip at the edge of the muscle below the plane of focus of the lens.

When the nerve endings upon the smooth muscle fibers of the sphincter muscle are examined, the investigator is immediately surprised at their enormous number. They are shown with the oil-immersion lens enlargement in figure 41. At the point where the parasympathetic fibers enter the plane of the muscle and terminate upon the fibers, the nerves are extremely fine, but some idea of their structure can be observed in figure 41. All the black dots seen in this photograph illustrate motor endings upon the muscle. Many of them occur in parallel rows. Occasionally the fibers break up into a group of grape-like endings supplying a number of muscle fibers. From this picture, it will be realized that every smooth muscle fiber probably receives a motor ending. Indeed, there is no place in which the great number of nerve endings upon smooth muscle can be more clearly observed than in the sphincter muscle of the iris.

It is not possible in mere descriptive anatomical studies of this type definitely to distinguish motor from sensory nerve fibers. However, on the basis of their structure, position and the complexity of their endings, it is probable that the large myelinated nerve fibers in the iris are sensory. This view has been confirmed by operative procedures isolating the nerves into different functional groups. In general, three types of endings have been seen in connection with these myelinated fibers. They appear to give rise to complex sprays of endings terminating in the superficial layers of the stroma of the iris wall. Secondly, there are endings which follow the course of blood vessels which are also of a complex variety. Thirdly, there appear to be endings in connection with the smooth muscle of the sphincter mechanism of the iris. These endings are much more complicated than the motor and are similar to the type of endings which we have previously described on the smooth muscle of the bladder wall and which we believe to be of the nature of stretch receptor mechanisms. It is very difficult to bring enough of the spray-like endings in the superficial layers of the iris into focus at one time to give any photographic demonstration of the nerve endings in this region. Portions of the complicated endings associated with the sphincter muscle are shown in two photographs (fig. 42 and 43). In figure 42, the arrangement of the smooth muscle fibers is indicated by the refractive differences. The group of black dots indicate a large nerve ending lying in the substance of the sphincter muscle and arising from a branch of a large myelinated fiber. A myelinated fiber is shown in figure 43 which gives rise to a group of complicated nerve endings lying on the smooth muscle cells of the iris sphincter.

The terminations of the myelinated fibers in association with blood vessels are extremely complex. Most of them lie near the free edge of the iris and run for a considerable distance over the muscle and around the margin of the iris, always in intimate relationship to the blood vessels. It is possible to show only a small portion of one of these endings in a photograph (fig. 44). Other portions of the endings of myelinated fibers on blood vessels are illustrated in figures 63 to 65. A peculiar swelling of the axone of a myelinated fiber is seen in figure 66.

The arteries and capillaries of the iris wall are abundantly innervated. It is interesting that no nerves have been seen following or ending upon the veins of the iris. For purposes of illustration, a small artery is shown in figure 45. Small nerve trunks accompany the artery on either side. Nerve fibers run over upon the arterial wall to end in fine dustlike endings there. One of these endings lies in very close connection with the nucleus of a smooth muscle cell in the wall of the artery. Indeed, this artery is well demarcated by the smooth muscle nuclei which are stained clearly.

In a previous paper, dealing with the urinary bladder (Langworthy and Hesser, 1940), it was pointed out that the capillaries of the bladder wall were abundantly supplied with nerves. This was also true of the capillaries of the iris. In earlier photographs of the iris in which the blood vessels had been injected with India ink, the pattern of the capillaries in the wall of the iris was shown. The nerves upon the wall of the small blood vessels and capillaries are illustrated in figure 50. It is immediately apparent that the pattern is the same

as that which was previously seen in the injected specimen of blood vessels, although in the present case the photograph was taken at higher power. The nerves, with this degree of magnification, appear as rows of dust-like granules forming a network which follows closely the capillary pattern. In focusing up and down upon the wall of the flattened iris, at least three of these capillary patterns can be observed. They differ slightly in the arrangement of the meshwork in each of the three layers. With the magnification seen in figure 50, the arrangement of the nerves cannot be made out well, but this is more perfectly seen under an oil-immersion lens in figures 51 and 52. In figure 52, nerve fibers run along the capillaries and, in one instance, the nucleus of a cell in the capillary wall is outlined. There is a nerve ending close to this nucleus. Another nerve fiber ends in a bulbous termination upon the capillary wall in the left portion of the photograph. This is the most perfect demonstration we have ever obtained of nerve endings upon capillaries.

In the photographs of the iris with the blood vessels injected with India ink, the arrangement of the blood vessels in the ciliary processes was demonstrated. It was observed that the ciliary processes were surrounded by a loose network of fibrous tissue. Figure 55 is a low powered photograph showing the nerves upon the blood vessels of a ciliary body. This photograph is given for purposes of orientation showing that the blood vessels of the ciliary body were well innervated. The more detailed arrangement of these endings upon the ciliary vessels will be described in a subsequent section. The stroma is abundantly supplied by nerves as shown in figure 56.

To summarize, we have endeavored to give a general idea of the nerve fibers and endings to be found in the iris of the albino rabbit. The parasympathetic fibers entering the iris from its peripheral portion take part in diffuse plexus arrangements, extend medially in the mesodermal layer and then dip downward to enter the sphincter muscle of the iris. The nerves have a finely beaded appearance. When the fibers enter the muscle, they branch diffusely into fine fibrils which run parallel to the muscle fibers and give rise to great numbers of dot-like endings upon the surface of the muscle fibers. These endings upon the muscle are extremely abundant.

Myelinated fibers branch diffusely and spread over the outer surface of the iris. At the point where they divide, there is an enlargement of the axones. These fibers give rise to plaque-like terminations in the mesoderm, complicated endings upon the blood vessels and large endings closely associated with the muscle fibers of the sphincter muscle.

Finally, there is a third group of fibers, which appear to be closely associated with the blood vessels. They extend along the walls of the larger blood vessels and form a plexus about the small arterioles and capillaries, so that there is a fine plexiform network through the substance of the iris. In focusing up and down through the thickness of the iris three fine vascular nerve plexuses can be recognized, varying somewhat in the arrangement and size of the meshwork. Terminations may be seen upon the arterial, arteriolar and capillary walls of the blood vessels. The veins do not appear to have associated nerves or nerve endings.

These sympathetic fibers also give rise to terminations in close contact with the chromatophores. In these preparations we have not been able to recognize endings upon the cells which are generally considered to make up the dilator muscle of the iris.

THE INNERVATION OF THE CHROMATOPHORES

The chromatophores may lie scattered in the stroma of the iris wall or gathered around blood vessels or nerves. They appear to be innervated by the fine, myelinated nerve fibers running through the stroma.

The general form of the chromatophores has been shown in previous pictures (figs. 27, 28, and 36). Figures 46 to 49 illustrate the relation of these pigment cells to the nerves. Nerve fibers usually make contact with the processes of the pigment cells. Thus the chromatophore in figure 46 shows branching processes which lie close to an unmyelinated fiber running above the cell. A nerve ending in rather close approximation to a process of a chromatophore is shown in figure 49. The nerve enters the photograph from the left side and gives rise to a bulbous termination just below the branching process of the pigment cell.

When chromatophores lie in the vicinity of small or large nerve trunks, the feet or branching processes of the pigment cells appear to reach out in the direction of the nerve fibers or trunk. This is shown specifically in figures 47 and 48. Two large myelinated fibers cross the field in 47 and the feet of the chromatophores extend into the vicinity of the nerve fibers. The same principle is illustrated in figure 48, where the foot of a chromatophore appears to touch small unmyelinated nerve fibers.

In summary, the chromatophores which are found in the mesodermal layer are commonest in the ciliary portion of the iris. They give rise to branching processes that are often oriented in relation to nerve fibers in the vicinity. Fine, unmyelinated nerve fibers give rise to small bulbous endings which lie in close contact with the processes or feet of the chromatophores.

INNERVATION OF THE BLOOD VESSELS OF THE IRIS

Knowledge of the method of innervation of the blood vessel walls has developed slowly. In Krogh's book on the anatomy and physiology of the capillaries, published in 1922, there is shown a photograph of the nucleus of a smooth muscle cell as an example of a nerve ending upon the wall of a capillary. The most important studies have been made by means of the vital methylene blue technique, inasmuch as it preserves the continuity of the vascular structures as cannot be done in sections. Thus, Woollard in 1926 showed complicated sensory endings close to the walls of the blood vessels. Hinsey (1928 and 1930) has given a detailed summary of the literature in addition to important personal observations.

In 1940, the innervation of the blood vessels of the urinary bladder was described (Langworthy and Hesser). Nerve trunks accompany the larger arteries. In their walls it is possible to demonstrate a complicated network of nerve fibers. Small nerves follow the capillaries and fine dots, representing nerve endings, appear in abundance upon the capillary walls. The nerves in the sub-

mucosa of the bladder are largely related to the walls of small arteries and capillaries. In addition, myelinated fibers give rise to complicated endings upon or in close vicinity to arterioles and it was suggested that these were sensory endings.

More information concerning the innervation of blood vessels was gained by study of the iris with the methylene blue technique. First, it was possible to observe clearly the arrangement of the nerve endings in small arteries, especially the circular artery derived from the long ciliary arteries. The analysis of a meshwork of nerve fibers resolved itself into a study of nerves and nerve endings having a very definite pattern in the muscular wall and in the endothelial layer of the artery. Nerves in the walls of blood vessels do not follow a syncytial pattern of a diffuse network but have a very definite arrangement. The appearance of nerves differs in the smooth muscle layer and in the endothelial layer of the blood vessel. These findings are illustrated in figures 58, 59 and 60. Figure 58 is a high power photograph of the arterial wall. Below the artery, slightly out of focus, may be seen the accompanying nerve trunk and branches extending up to the arterial wall. A similar branch is present above the blood vessel, but is so far out of focus that it is difficult to distinguish. The black lines and dots in the photographs represent the nerve fibers and nerve endings in the endothelial layer of the arterial wall.

The terminations in the endothelial coat of the blood vessel are shown under oil-immersion in figure 59. This demonstrates that they have a very definite pattern and end in small sprays upon the endothelium.

The arrangement of nerves and endings in the muscular coat is different in that the nerves tend to run parallel with the smooth muscle fibers in the wall and at right angles to the blood vessel. The nerve endings are shown with a high magnification in figure 60. The exact arrangements of the terminations upon the smooth muscle fibers are not shown here; it was possible to photograph endings close to the smooth muscle cells in smaller arteries. These will be described later.

The ciliary bodies are made up of a complex arrangement of blood vessels and it is difficult to follow the innervation of the vessels with any degree of exactitude. A portion of a ciliary process extending down toward the inner margin of the iris is shown in figure 55. The mesial prolongation of the ciliary process is at the right. The black dots indicate the nerve fibers and endings upon the ciliary vessels. With higher magnification it was possible to observe more clearly the relation of the nerves to the blood vessels of the ciliary bodies. Some of the capillaries contained red cells and nerves could be seen ending close to the nuclei of the endothelial cells of the wall. The nerves are abundant, not only in the ciliary processes, but also in the stroma. In general, the larger nerves run longitudinally toward the medial portion of the iris and the pupillary border (fig. 56).

It has been mentioned that nerves tend to follow smaller blood vessels, especially the capillary network, and give the appearance of a nerve syncytium in the wall of the iris. The pattern of these nerves and blood vessels varies in different layers of the iris. One of these patterns is shown in figure 50. Figure 51 is a higher magnification showing the arrangement of the nerves along the capillary walls. These fibers tend to give rise to endings upon the nuclei of cells in the

walls of the capillaries (figs. 52 and 53). These are probably the Rouget cells which have been described upon the capillary wall. One large termination can be seen at the left side of figure 52 in close contact with the nucleus of the cell.

It has been rather difficult to photograph the nerve endings upon the smooth muscle cells in the walls of small arteries. Figures 54 and 57 do show nerve fibers ending close to smooth muscle nuclei. They probably lie on the surface of the cytoplasm which has not taken the stain.

To summarize, many of the nerves in the iris run with and in intimate contact with the blood vessels. It has been possible to analyze the endings of nerve fibers on medium sized arteries (such as the circular artery of the iris), on arterioles, and on capillaries. In the circular artery of the iris, there are sprays of endings upon the smooth muscle in the wall. A more diffuse group of endings is present in the endothelial layer of the artery. In the arterioles, small endings can be seen close to the nuclei of the smooth muscle cells. These probably lie upon the surface of the cytoplasm which is not stained in the preparations. In the capillaries, endings are found in close contact with the nuclei in the vessel walls.

NERVE ENDINGS IN THE CHOROID COAT OF THE RETINA

In certain of the preparations a portion of the choroid layer of the eye remained attached to the peripheral portion of the iris and it was possible in the albino rabbits to see very clearly the arrangement of the nerves in the choroid layer. In this area the nerve fibers appear to be related closely to the blood vessels of the choroid. They are shown with low magnification in figure 61. At the points where the blood vessels divide there are cells with large nuclei and nerve endings may be seen on the surface of these nuclei. This is demonstrated in figure 62.

In summary, there are many nerves in the choroid layer of the eye. Most of them travel in close connection with the blood vessels of the choroid and end in contact with nuclei in the wall of the blood vessels. We have studied the capillary plexus in particular and an illustration is given of bulbous endings around a nucleus in a capillary wall.

THE ENDINGS OF MYELINATED FIBERS UPON THE BLOOD VESSELS OF THE IRIS

Already, it has been pointed out that the myelinated fibers in the iris appear to end in three ways; as free endings upon the surface of the iris, as complicated endings upon the smooth muscle of the sphincter and in even more complicated endings upon the walls of the blood vessels. There are many reasons to believe that these myelinated fibers are sensory and that the endings upon the blood vessels are sensory terminations which control the tone of the smooth muscle in the blood vessel walls.

These endings are so large and complicated that it is difficult to illustrate them in photographs and to describe them very clearly (fig. 44). However, it has become apparent that the fibers give rise to endings close to the nuclei of cells along the walls of capillaries and possibly of arterioles. The endings are larger than those of the unmyelinated fibers which probably serve a motor function. For this reason, they can easily be recognized where they occur.

A portion of one of the endings is shown in figure 63. It shows a myelinated fiber giving rise to large sprays of bulbs, extending out in all directions along the course of blood vessels. These fibers show areas of extreme swelling along the course of the fiber as well as the sprays of endings (fig. 38). One of the swellings as seen with the oil-immersion lens is illustrated in figure 66. Figures 64 and 65 indicate the terminations in relation to the walls of the blood vessels and the nuclei found in the walls. Here it is shown very clearly that endings are present close to the nuclei of the endothelial cells.

In summary, myelinated fibers end in close connection with arterioles and capillaries, particularly close to the pupillary margin of the iris. The endings are very large and diffuse and the terminal end bulbs are somewhat larger than the endings of the unmyelinated fibers. It is probable that the myelinated fibers give rise to sensory endings. The terminal plaques are often in close contact with the nuclei of the endothelial cells in the capillary walls. The myelinated fibers often show localized enlargements of the axones which are usually in contact with the endothelial cells in the wall of a capillary or small artery.

SECTION OF DIFFERENT NERVES SUPPLYING THE IRIS TO DETERMINE THE ORIGIN OF THE FIBERS

To obtain further information concerning the origin of the nerve fibers which have been described in the iris, an attempt was made to cut the different nerve trunks and stain the iris after time had been allowed for degeneration of the nerve fibers and their endings. The postganglionic sympathetic neurones theoretically can be severed by removal of the superior cervical ganglion. Similarly, extirpation of the ciliary ganglion should cut the postganglionic parasympathetic neurones. The ophthalmic branches of the trigeminal may be cut in the orbit.

The superior cervical ganglion was exposed and removed under aseptic precautions in 20 rabbits. After a period varying from 7 days to 4 months, the eyes were stained as already described and the iris upon the operated side, compared with that of the normal side which served as a control. This control method is not entirely satisfactory due to vagaries in the stain, but after comparing a large group of animals it gives conclusive information. Following this operation, there was undoubtedly a great decrease in the number of nerve fibers supplying the blood vessels, including those of the ciliary body. The nerves in the stroma of the iris and those ending around the chromatophores were also decreased in number. No change could be seen in the number of nerve endings upon the sphincter muscle of the iris. The myelinated fibers and their complex nerve endings remained normal in number and appearance after removal of the superior cervical sympathetic ganglion. The extent of the loss of unmyelinated nerve fibers after this operation varied to a certain extent in the different cases. It was concluded that in all probability there are many postganglionic sympathetic ganglia distal to the superior cervical ganglia, making it impossible to remove all the postganglionic sympathetic fibers to the eye by this operation. Moreover it is possible that a number of sympathetic fibers reach the eye along

the blood vessels and are not associated with cells in the superior cervical sympathetic ganglia.

In the vitally stained preparations it was never possible to identify the thin layer of cells which has been designated as the dilator muscle of the pupil. For this reason we have no specific information concerning its innervation. It can be said that the iris stroma of which this layer is a part showed a diminution of nerve fibers after the superior cervical ganglion had been removed.

Another operation removed the ciliary ganglion in the orbit. The number of nerve fibers cut in addition to those connected with the ciliary ganglion was variable. The long ciliary sympathetic nerves which lie close to the ciliary ganglion may be removed or left intact. Similarly the sensory fibers from the trigeminal nerve which lie close by may or may not be severed. In all cases after removal of the ciliary ganglion, there was a total loss of nerve fibers upon the sphincter muscle of the iris and it may be assumed that these fibers are all of parasympathetic origin. In some cases, the large myelinated fibers which reach the iris were lost entirely but in many cases they remained in normal number. Since they were not involved at all after removal of the superior sympathetic ganglion and not injured in every case after removal of the ciliary ganglion, it is probable that they are sensory fibers derived from the trigeminal nerve which runs close to the ciliary ganglion, and are not derived from the oculomotor nerves and do not run to the central nervous system along with the parasympathetic fibers.

Often, in removal of the ciliary ganglion, the sympathetic fibers in the long ciliary nerves were severed at the same time and there was almost a complete loss of unmyelinated nerve fibers along the blood vessels and in the stroma of the iris. A more complete denervation of the stroma of the iris and the blood vessels was obtained after removal of the ciliary ganglion including the long ciliary nerves, than ever was achieved after removal of the superior sympathetic ganglion. This supports the suggestion that there are sympathetic ganglia distal to the superior cervical ganglion.

After removal of the superior cervical ganglion there were still many fibers in the stroma which gave off endings in close contact with the cytoplasm of the chromatophores. However, after removal of the ciliary ganglion and the long ciliary nerves at the same time, these fibers in the stroma were largely lost. Under these conditions, the chromatophores tended to lose their branching processes and the cytoplasm became round. This is a characteristic change which follows denervation of the pigment cells.

There appear to be changes in the arteries of the iris after the sympathetic fibers have been sectioned by removal of the nerves in the posterior portion of the orbit. It was only under these conditions that the vessels were free from nerve fibers. In the region of the arteries after the nerves had degenerated, there were a number of small branching cells situated in the adventitia of the vessel. These cells have previously been described by Woollard (1926) and others. They are present normally but become conspicuous after the nerves are lost. Their function has never been clearly understood. It also appeared

that there was a change in the smooth muscle cells in the walls of the arteries after denervation. The nuclei which stained in these preparations appeared to be much thinner and smaller than in the normal preparations. It would appear that there was a certain atrophy of the muscle fibers as manifested by the appearance of the nuclei. In the normal animal the nuclei of the smooth muscle cells and sometimes their cytoplasm stained in the preparations with methylene blue. After section of the nerve, the endothelium in the artery often stained too, so that it could be made out quite clearly. The staining was diffuse involving the cytoplasm of the endothelial cells.

In summary, removal of the ciliary ganglion removes all the nerves ending upon the sphincter muscle of the iris. The myelinated nerve fibers in the iris may or may not be injured by the removal of the ciliary ganglion. It is thought that they arise from the trigeminal nerve, run close to the ciliary ganglion and are often injured by the operation. The nerve fibers to the blood vessels and stroma are never completely removed by excision of the superior cervical sympathetic ganglion. It is probable that there are other postganglionic sympathetic cells, peripheral to this ganglion. Removal of the ciliary ganglion of the eye which may destroy the long ciliary nerves at the same time, in certain cases caused a loss of almost all the nerve fibers to blood vessels and stroma. When the chromatophores in the iris were denervated, they tended to lose their branching processes and to become circular in form. After denervation of the arteries, the smooth muscle nuclei in their walls appeared to become thinner and more gracile as if there had been some atrophy of the smooth muscle cells themselves.

DISCUSSION

The blood supply of the iris has been discussed as seen in preparations with the blood vessels injected with India ink. The astonishing complexity of the vascular pattern brings forward the possibility that contraction and dilatation of the vessels have an important part in the control of pupillary size. This is by no means a new suggestion but it has been lost sight of in recent years.

It will be remembered that in certain animals a unique method of pupillary closure has been developed in which an operculum or curtain falls over the pupillary aperture. This operculum is composed of blood vessels and fibrous tissue with no smooth muscle, but in response to stimuli the blood vessels in an operculum dilate, making the structure expand to close the pupillary aperture. Thus, in certain forms an effort has been made to produce a method of pupillary closure which depends on no action of smooth muscle but upon the dilatation and contraction of the blood vessels.

The position of the so-called dilator muscle in the iris has been described and the cells which compose it are shown in photographs. There is no well defined outer limit to the sphincter muscle of the iris. This muscle is thick near the pupillary margin, but laterally the fibers become more tangential and spread out toward the periphery of the iris. In this lateral margin, the fibers tend to mingle with those which make up the so-called dilator muscle of the iris. The dilator muscle layer is very thin and consists of small strands of fibers which give some-

what the appearance of smooth muscle. It is possible that this is not a distinct layer of dilator muscle at all but is composed of undifferentiated cells which more medially have been developed to form the sphincter muscle of the iris.

Certainly the importance of the blood supply of the iris in controlling the size of the pupil should be subjected to further experimental studies. It would be interesting to have data as to the changes in the pupil produced by raising or lowering the blood pressure. Some evidence on this point has been given in the pages which preceded. It would appear that the vascular supply is capable of influencing the width of the iris and size of the pupil. This factor is little affected by reflex changes. The movements of the sphincter muscle either in contraction or relaxation play upon a rather constant dilator force producing a change in the size of the pupils. After the sympathetic fibers have been cut, the dilator power of the pupil is somewhat decreased. The blood vessels still have a certain passive elasticity related to the smooth muscle in their walls and to the general circulation but have lost their nervous control. With the sympathetic fibers intact, there is a direct nervous control of the blood vessels in the iris wall itself.

There appears to be no antagonism between the sympathetic and parasympathetic control of the iris affecting the size of the pupil. The sympathetic influence is a more or less continuous one which is affected very little, if at all, by any reflex activity directed toward the iris itself. Possibly painful stimulation tends to stimulate the sympathetic influences on the iris, but light and accommodation apparently exert no effect through the sympathetic pathway. The sympathetic innervation gives a certain dilator tendency to the pupil against which phasic movements of the sphincter muscle in the direction of dilatation or contraction are allowed to play continually. Indeed, in no part of the body is the antagonism between sympathetic and parasympathetic fibers clearly demonstrated, and this theory which has been maintained for many years must be put in the discard.

Certainly the pupils are extremely responsive to painful stimuli and these appear to exert a great effect upon the pupillary size. The painful or emotional stimuli are probably effective largely through the parasympathetic innervation of the iris, although there may be influence through the sympathetic system, possibly as a general adrenalin reaction. Pain is known to produce a constriction of peripheral blood vessels and this response is probably mediated by sympathetic fibers.

The innervation of the iris is unusual in that the sensory fibers apparently run to the central nervous system with the trigeminal nerve, quite separate from the sympathetic and parasympathetic fibers. Thus there are three great groups of fibers innervating the iris, sympathetic, parasympathetic, and sensory. This demonstrates in a rather clear way that the autonomic is a purely motor system made up of afferent fibers leaving the spinal cord and brain stem from certain definitely localized areas. Workers have spoken of the sensory fibers innervating smooth muscle and glandular tissue as autonomic sensory fibers and discussed a sympathetic or parasympathetic reflex arc. However, as can be demonstrated in the case of the iris, sensory fibers from all portions of the body tend to act

through the parasympathetic pathway to produce a change in the pupillary size. Therefore, there is no reason why the sensory fibers from the iris should be designated as autonomic fibers and it would be better to speak of them merely as sensory fibers.

The beaded appearance of many of the nerve fibers in the iris stained with methylene blue has not been explained. Many of the fibers showing this beaded appearance are unmyelinated or finely myelinated. It might be considered that this picture is due to post mortem changes. However, the preparations were not studied under the microscope in their fresh condition. The staining of the iris scarcely consumed over 15 minutes. After the membrane had attained a proper blue color, it was immediately placed in the fixative and the preparations were studied only after they were fixed and cleared. It must be assumed, therefore, that the tissue had little time for any post mortem changes before fixation occurred.

One gains the impression that there are huge numbers of motor endings upon the sphincter muscle of the iris and it seems probable that each smooth muscle cell in the sphincter muscle receives a separate innervation. For many years it has been thought that the autonomic endings on smooth muscle are few in number. This error was probably dependent upon the difficulty in staining the great number of endings. In order to overcome this difficulty, Rosenbleuth and Rioch (1933) and Rosenbleuth and Cannon (1936) have suggested a chemical mediator spreading out from the few nerve endings on the smooth muscle to insure uniform contraction of the muscle. The large number of endings upon the sphincter muscle is demonstrated in well stained preparations, so that it is apparent that no great diffusion of the chemical mediator would be required.

There are enormous numbers of dot-like endings and it is difficult to make an analysis of their arrangment. The dots also appear to occur along the course of the nerve fibers and one wonders whether these are nerve endings or are related to the fiber itself. In places where the network can be analysed more clearly it is seen that small branches are often given off, giving rise to endings as dots and bulbs.

Indeed, the nerve fibers and nerve endings are so abundant and complex in the case of the blood vessels of the iris, that any understanding of their arrangement in connection with the wall of the artery is difficult. It is always tempting to assume with Boeke that there is a nerve net with anastomosing fibers and that the endings of the fibers cannot be located and are indeed not necessary for contact with the effector organ. However, it has been possible to show that the nerves give rise to bulbous terminations close to the nuclei of cells in the walls of small arteries and capillaries. Photographs are shown of nerve endings upon the walls of arteries and capillaries as stained vitally with methylene blue. It is probable that if the preparations were not as complicated and fewer fibers were stained, it would be possible to follow all the fibers to terminations upon the walls of the vessels and demonstrate that no network is present in the arterial wall.

Agababow observed two plexuses on the outer wall of the iris; one near the endothelial surface and one more closely connected with the blood vessels. He

believed that the outer one was sensory and the inner one motor. It is quite clear that the myelinated fibers of the iris give rise to great numbers of endings upon the surface of the iris in the mesodermal layer. The largest and most complicated terminations are connected with the blood vessels near the pupillary margin or lie among the fibers of the sphincter muscle. However, it is probable that these medullated fibers give rise to numbers of subendothelial terminations over the whole surface of the iris and some of these disk-like endings have been identified. It is difficult, however, to bring any number of them into focus at the same time with the high powers of the microscope.

Woollard has given an excellent description of sensory endings upon blood vessels in the extremities. He has described plume- and brush-like endings upon the blood vessels and other branches of the myelinated sensory fibers extending away from the blood vessels to end in encapsulated corpuscles or on fat cells in the vicinity. In the preparations of the iris we have observed very complicated endings of myelinated fibers upon the blood vessels near the pupillary border which we suggest are proprioceptive in type. It would be interesting to know whether the same myelinated fiber may give rise to a proprioceptive ending upon the muscle and an ending upon the wall of blood vessels. Woollard believed that there was very little innervation of the capillary wall.

As a result of study of the urinary bladder and now of the iris, it seems probable that almost all of the capillaries of the body are supplied with unmyelinated nerve fibers giving rise to bulbous terminations around the nuclei of the endothelial cells in the capillary walls. Certainly, in the iris, it is possible to distinguish at least three plexuses of nerve fibers at different levels of the iris wall. These plexuses follow the vascular pattern very closely. They are found not only upon the arteries and arterioles but also upon the capillaries in these three different layers of the iris wall.

The studies of the iris again show the great difficulty in producing a complete denervation of the blood vessels in any area of the body. In the urinary bladder, a number of years ago, an endeavor was made to cut all the nerves ending upon the blood vessels in the bladder wall. This was almost impossible of accomplishment without isolating the bladder completely from other nearby structures. Other workers have had the same difficulty in relation particularly to the nerves upon cerebral blood vessels. Removal of the superior cervical ganglion is said to have almost no effect in removing the vascular plexus upon the blood vessels of the brain. Similarly in the case of the iris, removal of the superior cervical ganglion causes only a minor loss of nerves upon the blood vessels, after time had been allowed for degeneration of the sympathetic fibers. However, removal of the ciliary ganglion in the orbit, including the long ciliary sympathetic nerves at that level, usually causes a fairly complete denervation of the blood vessels. It is possible, of course, that there are postganglionic sympathetic cells distal to the superior cervical ganglion. Another obvious possibility is that some of the nerves to the blood vessels run to the eye by way of the oculomotor nerve and the ciliary ganglion.

After the nerves to the blood vessels of the iris have been sectioned and degenerated in large part, it was possible in the preparations stained with

methylene blue to observe some changes in the smooth muscle nuclei of the arterial walls. The nuclei became thinner and more gracile which suggests that there was a moderate atrophy of the smooth muscle in the walls of the arteries.

Pollack (1924) studied the nerves in the iris stained with methylene blue. He believed that a peripheral plexus containing nerve cells existed both in the sphincter and dilator muscles of the iris. The plexus lay between the individual cells of the muscle and was made up of fibers of extreme tenuity. The plexus persisted after isolation of the iris from the central nervous system by removal of the ciliary ganglion and the superior cervical ganglion. It was therefore regarded as of the same nature as the plexus of Auerbach and Meissner in the intestines. This shows again the difficulty encountered in removing completely the nerve supply to the iris by section of the nerve trunks.

SUMMARY

Recent physiological experiments show that most, if not all, reflex responses of the iris are controlled through the parasympathetic pathway. There is some question whether pain will still cause dilatation of the pupils reflexly through the sympathetic pathway, after the parasympathetic fibers are cut and the pupil constricted somewhat with eserine. Even if this response to pain is present, it may be a reaction to adrenalin, and not a reflex through the sympathetic system.

It was realized from early times that the iris is an extremely vascular membrane and for many years it was thought that changes in the size of the pupil were dependent upon vascular changes in the iris. Thus constriction of the vessels of the iris produced a dilatation of the pupil and dilatation of the vessels, a constriction of the pupil. A sphincter muscle was later observed in the iris, but it was still thought that the dilatation was controlled by vascular changes. It was only at the end of the last century that Langley and Anderson postulated the presence of a dilator muscle. Anatomists still are not in agreement concerning the presence or position of this dilator muscle. It is supposed to be a thin membrane differentiated from the outer layer of the primitive optic cup.

In preparations of the iris showing the blood vessels injected with India ink, it is possible to see the marked vascularity of the structure and the complicated vascular pattern of the ciliary bodies. All the arteries of the iris have a curved course so that they look like coiled springs. This permits them to conform to widening or narrowing of the membrane. The medial prolongations of the ciliary bodies extend far toward the pupillary margin and appear capable of varying their conformation dependent upon the amount of blood contained in the vessel and the contraction or relaxation of the musculature of their walls. In preparations stained with methylene blue, it was possible to observe the heavy muscular coat which is found even in the small arteries. This muscle is not regularly placed along the course of the wall and is most marked at the place where the blood vessels curve. It is possible that contraction or relaxation of this muscle would have a marked effect on elongating or shortening the arteries of the iris.

It has been further shown that three groups of nerve fibers innervate the iris; parasympathetic, sympathetic and sensory. The sensory axones are myelinated, the cells of origin probably lie in the trigeminal ganglion, and the fibers reach the

eye with the ophthalmic branch of the trigeminal nerve. These medullated fibers give rise to three types of nerve endings in the iris. There are disk-like endings in the mesoderm in the outer surface of the iris, complicated endings lying between the smooth muscle fibers of the sphincter muscle, and large and diffuse terminations upon the blood vessels near the pupillary margin. It is probable that the endings embedded in the smooth muscle of the sphincter are proprioceptive and the endings upon the blood vessels have a part in controlling the tone and contraction of the blood vessels in the iris. Injury of these sensory endings in the smooth muscle of the iris and upon the blood vessels may cause a profound physiological disturbance but its nature has not been recognized up to the present time.

The parasympathetic fibers leave the central nervous system by the oculomotor nerve and the postganglionic fibers arise in the ciliary ganglion. There are about twenty short ciliary nerves whose fibers eventually innervate the sphincter muscle of the iris. The sympathetic fibers arise from the upper thoracic portion of the cord and many of the postganglionic fibers originate in the superior cervical ganglion. However, removal of the superior cervical ganglion by no means causes a loss of all the sympathetic fibers to the eye, so that it must be assumed that postganglionic cells lie more peripherally. The sympathetic fibers end upon the blood vessels of the iris, innervate the chromatophores and end in the iris stroma.

It has not been possible to recognize the so-called dilator muscle in the vitally stained preparations, so we cannot speak of its innervation. We have preferred to lay emphasis upon the enormous vascular supply of the iris innervated by sympathetic fibers and the possibility of changes in the width of the iris and subsequent variations in the pupillary aperture produced by the contraction or dilatation of these vessels. This paper by no means denies the possibility of a dilator muscle in the iris. This muscle, if present, seems poorly developed. It has been impossible to study its innervation. The blood vessels with the muscle in their wall are well developed and seem adapted to serve as a dilator mechanism.

While injecting the iris with ink or with dye, sectors of the membrane often stained before other portions. These areas became wider and the adjacent pupillary edge became flattened, producing an irregularity of the pupil. Irregularity of the pupil can be produced by an unequal dilatation of the blood vessels of the iris, so that the blood vessels are filled in one sector and not in another. By raising or lowering the pressure of the injecting fluid, it is possible to influence in a marked way the contraction or dilatation of the pupil. When the pressure is high, the pupil becomes small, but when the pressure of the injecting fluid is lower, the pupil immediately becomes larger in size. This demonstrates physiologically the effect of the blood vessels upon the expansion or contraction of the iris and the changes in pupillary size. Moreover, when the blood vessels were well filled with the dye or with the ink, the pupil was always small. The iris has more or less of the structure of an erectile tissue. Vasodilatation produces a small pupil and vasocontraction produces a large one.

The blood vessels of the iris can change their status as regards constriction or

dilatation by three possible mechanisms. The actual nerve supply to the muscle of their walls can produce active changes. After the nerves are cut, the smooth muscle can still respond to drugs or hormones. The filling of the vessels can also be influenced passively by changes in the general circulation. Physiological studies of the iris have stressed an active force toward dilatation under sympathetic control and a passive force which is present after the sympathetic fibers are cut. The latter is sometimes described as elastic recoil.

The nerve fibers ending upon the arteries and capillaries of the iris are by no means lost after removal of the superior cervical ganglion. Large numbers remain. Removal of the nerves in the orbit including the long ciliary nerves, ciliary ganglion and trigeminal fibers causes almost a complete loss of nerves to the blood vessels. Two explanations need to be considered. The unmyelinated vascular fibers are sympathetic and postganglionic sympathetic cells lie distal to the superior cervical ganglion, or there are large numbers of unmyelinated vasodilator fibers in the parasympathetic pathway. The latter possibility is a fascinating one inasmuch as stimulation of the parasympathetic fibers would cause both contraction of the constrictor muscle and vasodilatation, both tending to make the pupil smaller. On the other hand, there has never been any adequate proof of vasodilator nerve fibers.

Part III. Theoretical Discussion of Abnormalities of the Pupils Observed in Man

Introduction

Naturally, it is tempting to apply the anatomical findings to an explanation of abnormalities of the pupils found in man. This discussion will necessarily be of a theoretical nature. It is customary to explain the pupillary changes in syphilis as due to lesions in the central nervous system. On the other hand, a few authors have inclined to ascribe the changes in the pupils to abnormalities in the orbit, either in the ciliary ganglion or in the iris itself. Before the merits of these theories can be considered, it is necessary to gain some understanding of the control by the central nervous system of the iris and of the pupil through sympathetic and parasympathetic pathways.

The Control by the Central Nervous System of the Sympathetic Innervation of the Iris

It is well known that section of the cervical sympathetic trunk produces a Horner's syndrome. This consists of miosis, enophthalmos, ptosis, anhidrosis, and flushing of the face. If the sympathetic fibers are stimulated, the converse condition is produced. Then there appear mydriasis, exophthalmos, widening of the palpebral fissure, sweating and pallor of the face. Pressure upon the sympathetic trunk in the upper portion of the thorax or neck, may first lead to irritation and finally to paralysis of the sympathetic fibers. If a Horner's syndrome is produced, the abnormalities are most marked directly after the fibers are severed and tend to regress even before the nerve fibers regenerate.

Cobb and Scarlett (1920) found the most severe examples of Horner's syn-

drome are those due to injuries of the seventh and eighth cervical and first thoracic nerve roots. Less severe symptoms are produced by injury of the cervical portion of the sympathetic trunk and least pronounced changes follow injury of the spinal cord.

It will be remembered that the sympathetic fibers travel upward in the cervical sympathetic trunk, then join the carotid arteries and finally reach the trigeminal ganglion. They run to the eye with the ophthalmic branch of the trigeminal nerve. The sympathetic fibers may be injured in the region of the trigeminal ganglion, producing the paratrigeminal pupillary syndrome described by Raeder (1924). This was further discussed by Monrad-Krohn (1931).

In recent years it has become apparent that there is an ipsilateral control of the sympathetic innervation of the iris from the region of the hypothalamus. Thus, Karplus and Kreidl (1909) were able to stimulate an area in the region of the hypothalamus which produced maximal dilatation of the pupils. Excitation of this area upon one side of the brain produced dilatation of the pupil upon the same side. The response could not be obtained if the cervical sympathetic trunk had been cut. On the other hand, section of the oculomotor or trigeminal nerves did not alter the response.

Huet in 1911 studied the retrograde degeneration resulting from extirpation of one superior cervical ganglion in newborn kittens. He observed a loss of cell and fiber elements beneath the third ventricle in the central gray matter of the homolateral side. The habenular nucleus occupied a lower position on this side, due to the atrophy of the adjacent tissue. He believed that these findings supplied the anatomical basis for the physiological experiments of Karplus and Kreidl.

Beattie, Brow and Long (1930) demonstrated by the Marchi method an uncrossed pathway arising in the hypothalamus and running into the spinal cord. At the upper end of the midbrain, the fibers lie ventral to the posterior commissure. Below this level they divide into two groups. One lies in the lateral portion of the midbrain and descends in the reticular formation to the pons and medulla. The other fibers travel downward in the medial longitudinal fasciculus. The hypothalamus apparently influences the pupil only through this sympathetic pathway.

Numerous workers have observed that tumors or other lesions in the region of the midbrain or medulla may produce Horner's syndrome. Merritt and Finland (1930) described six patients with softening of the lateral portion of the medulla, due, as they believed, to occlusion of the artery of the lateral sulcus. The Horner's syndrome was noticed in every instance. They gave some information concerning the permanence of this sign. In the first case, the miosis became less noticeable after a few weeks but was present until death three months later. In the second, the eye signs disappeared three and a half months after the vascular accident. The third was discharged after five weeks with a miosis still apparent. The fourth case died ten days after admission with the miosis present at the time of death. During the ten weeks that the fifth case was under observation, the Horner's syndrome still persisted. The eye was removed

in the sixth case a month after the vascular accident, terminating the observations. It appears in these cases that the pupillary changes tend to diminish and possibly to disappear if the patient survives. They may, however, be evident for months.

Fractures of the cervical and upper thoracic vertebra not uncommonly injure the spinal cord and produce Horner's syndrome. The injury either interrupts the efferent pathways descending from the hypothalamus or damages the preganglionic cells in the ventrolateral gray columns of the upper thoracic cord. Foerster (1928) presented evidence that the pathway from the hypothalamus runs in the anterolateral column in the cervical region. He noticed that it is often destroyed by a chordotomy operation performed at this level for the relief of pain.

In summary, then, there is a pathway from the hypothalamus to the upper thoracic portion of the cord which is uncrossed and which apparently controls the sympathetic activity of the pupil. After this pathway is injured, there is a Horner's syndrome, but the symptoms tend to regress and to disappear if the patient survives. In the region of the midbrain, the pathway lies ventrolateral to the central gray matter around the aqueduct of Sylvius.

THE CONTROL BY THE CENTRAL NERVOUS SYSTEM OF THE PARASYMPATHETIC INNERVATION OF THE IRIS

At the present time, little is known about the reflex arcs in the brain which control the pupils by the parasympathetic pathway. It is probable that influences from almost any part of the central nervous system can produce changes in tone and contraction of the sphincter muscle. Thus painful stimulation of any portion of the body will cause the pupil to dilate; it is probable that this reflex acts largely through the parasympathetic pathway.

It is universally accepted that stimulation of a localized area in the occipital lobe of the brain causes a constriction of the pupils. The pathway from the occipital lobe is thought to permit voluntary control of accommodation.

Smith (1936) found that stimulation of an area in the cerebral cortex of the monkey medial to the inferior precentral sulcus produced a group of movements which together similate an awakening response. Both eyes while opening slowly deviate toward the contralateral side. The pupils dilate and the eyes close and open several times. There is a complicated pattern response involving both striated and smooth muscle.

Stimulation of almost any portion of the surface of the cerebral cortex or indeed, of the underlying structures in the forebrain will produce a dilatation of the pupil. Probably this is a pain response related to excitation of blood vessels or meninges.

Following a cerebral vascular accident producing hemiplegia, there is sometimes a larger pupil on the paralysed side for the first few days. Later, the pupils tend to be equal in size. This inequality of the pupil is by no means present in every case. In this laboratory, it was found that in nine of twelve cats, after removal of the left cerebral motor cortex, the pupil was larger upon

the right side for a period from ten to twelve days. Thereafter, the pupils appeared equal.

Aring and Merritt (1935) found that unilateral dilatation of the pupils occurred in twenty-five per cent of cases of cerebral hemorrhage and 7.4 per cent of cases of cerebral thrombosis. The pupil was dilated on the hemiplegic side of the body.

In summary, it is probable that reflex arcs through almost all portions of the central nervous system control the tone and contraction of the sphincter muscle. These responses have never been demonstrated in detail. Painful stimuli will cause the pupils to dilate and it is probable that this response travels along the parasympathetic pathway. Stimulation of the occipital cortex causes a constriction of the pupils. It is supposed that a pathway for voluntary accommodation arises from this region. Stimulation of almost all portions of the surface of the cerebral cortex causes the pupils to dilate. It seems likely that this is a pain response, due to stimulation of the overlying meninges or blood vessels. A reflex dilatation of the pupils has been obtained from the motor area. Injury of the cerebral motor cortex often causes a transient dilatation of the pupil upon the opposite side of the body.

PUPILS IN CASES OF CHRONIC ENCEPHALITIS WITH A PARKINSONIAN SYNDROME

Little has been written concerning the pupils in patients with a Parkinsonian syndrome produced by chronic encephalitis. The pupils are usually already dilated with hyoscin when the patient is seen, making observations impossible. Moreover, in order to recognize changes in the pupil, it is important that the Parkinsonian symptoms should be predominantly unilateral. Fortunately this is occasionally the case.

When the Parkinsonian picture is marked only upon one side of the body, the pupil is smaller upon the same side. It reacts well to both light and accommodation. Indeed the pupil upon the abnormal side often seems to react more quickly than the opposite pupil. Possibly this can be explained by the fact that the smaller pupil places the sphincter muscle in an optimal position to respond.

It may be assumed that the smooth muscle in this disease shares the same general increase in tone shown in striated muscle. This has been shown in the case of the urinary bladder. Many patients with the Parkinsonian syndrome complain of frequency of micturition because the bladder is contracted, probably due to the increased tone in the wall. It would appear that the same condition occurs in the smooth muscle of the iris which has increased tone or rigidity, thereby maintaining the small size. There is no fundamental difficulty with muscular contraction either in smooth or striated muscle in this disease.

In cases of Parkinsonian syndrome, it may be assumed that the disturbance of the iris is due to injury at a suprasegmented level. Changes in the pupils in cases of hemiplegia have already been mentioned. Probably smooth muscle is influenced by reflex stimuli from all portions of the central nervous system, just as is the striated muscle of the body. This has been demonstrated more clearly in studies of the urinary bladder (Langworthy, Kolb and Lewis, 1940).

THE PUPILS DURING SLEEP

During sleep, the pupils are moderately contracted. They approximate the size of the pupils after section of the sympathetic fibers.

The explanation for the contraction of the pupils in sleep has never been satisfactory. Ury and Oldberg (1940) suggested that the sphincter muscle of the iris was controlled through the parasympathetic fibers at different reflex levels of the central nervous system. During waking hours the cortical control tends to produce a dilatation of the pupil. The authors suggested that there was a mechanism in the midbrain which controls tone in the sphincter muscle and is under inhibitory control from the cortex. They felt that during sleep the cerebral influences are diminished and the pupil becomes small under the increased influence of tone mediated through reflex centers in the midbrain.

It would seem more logical to believe that the size of the pupil during sleep represents the resting state of the iris and the maximal relaxation of all the structures in its wall including the sphincter muscle. Certainly sleep does not produce an increased tone in the striated muscles of the body, but on the other hand they show a relaxation. It would not be expected that a completely dilated pupil, but a point somewhere between dilatation and constriction would represent a resting position for the sphincter and dilator influences of the iris. The dilator mechanism must be at rest at the same time as the sphincter. The tone of the blood vessels tending to produce dilatation of the pupil during the day is relaxed. The pupil assumes the size found after section of the sympathetic fibers which represents the amount of dilatation of the arteries and blood vessels which is maintained by conditions of the general circulation with nervous control of the iris vessels removed. Thus, both section of the sympathetic fibers and sleep tend to place the iris in a resting posture.

The sphincter muscle of the iris can contract most efficiently if the pupil is moderately small. In an individual with large pupils indicating some general bodily tension, the pupils react to light and accommodation through only a small range. They do not become constricted to a minute size even under the stimulus of strong light. The constrictor movements, therefore, are less efficient when the pupil is greatly dilated.

During sleep, then, both the constrictor and dilator mechanisms of the pupil are in a state of relaxation producing the relatively small pupil observed in the sleeping individual. Indeed, it would be difficult to think that the sphincter muscle of the iris during sleep has increased tone due to overactivity of midbrain mechanism and the dilator mechanism of the pupil has decreased tone allowing the pupil to constrict.

If the relatively small pupils during sleep represent the pupil at rest, during the waking period there are powerful stimuli tending toward dilatation of the pupil which are active during the entire waking period. This tendency toward dilatation probably consists of at least three parts. First there is vasoconstriction of the iris vessels. Second, changes occur in the general circulation. Finally constant reflex relaxation through the parasympathetic mechanism acts upon the sphincter muscle of the iris.

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THE PUPILS IN OLD AGE

In early adult life the pupils tend to be of large size. As age increases they gradually become smaller and in people far advanced in life may be miotic. Indeed, they are sometimes pin-point. However, they react promptly to both light and accommodation, although this reaction is necessarily of small amplitude. The dilator mechanism becomes senile earlier than the constrictor. Possibly the small size of the pupils in old age is dependent upon the general relaxation of the iris tissues similar to that which is found elsewhere in the body in senesence. It may be related to a sclerosis of the blood vessels of the iris and a diminution of tone in their walls. Thus the dilator mechanism becomes less efficient, allowing the sphincter muscle to take a dominant part in pupillary control.

The dilation of the pupils in young adults is dependent not only upon a relaxation of the sphincter muscle of the iris, but also a positive action in the opposite direction produced by pull of the blood vessels or possibly by a dilator muscle. If it is dependent upon a dilator muscle, this muscle should be as efficient in old age as is the sphincter muscle. However, in old people the blood vessels would tend to lose their elasticity. They no longer act as spiral springs but become straightened. Unless the elastic recoil of the pupil is present, relaxation of the sphincter muscle does not produce satisfactory dilatation of the pupils.

Thus, the sphincter muscle of the iris in old age tends to hold the pupils tightly closed. Relaxation is poor inasmuch as the elastic recoil of the iris is lost. The sphincter muscle acts more efficiently when the pupil is small than when the pupil is large. Thus a good reaction of constriction to light and accommodation is still present although the range of the movement is small. The small pupils in old age tend to be somewhat irregular. Indeed, all very small pupils tend to show some irregularity in the margin of the iris.

THE PUPILS IN DEATH

Immediately after death, the pupil tends to dilate to maximal size. There is a complete loss of tone in the fibers of the sphincter muscle. On the other hand, there is a constriction of the iris blood vessels and a drainage of blood from the peripheral structures of the body into the venous system. The elastic recoil of the dilator mechanism is active and the sphincter muscle is entirely paralysed. This fact, in itself, seems to argue against the presence of a specific dilator muscle in the eye. If a dilator muscle were paralysed by the death of the animal, the pupil in death should be midway between dilatation and contraction, inasmuch as two opposing muscles are put out of function at the same time. If the dilator mechanism is related to the blood supply, a loss of nervous control of the arteries and collapse of the circulation at death would tend to pull the pupil open.

THE PUPILS IN CASES OF OPTIC ATROPHY

When the optic nerve is sectioned upon one side, the blind pupil becomes dilated. It still reacts to consensual light and to accommodation reflexes. If both optic nerves are sectioned, both pupils become large. They still react to

accommodation. The sphincter muscle which reacts reflexly to light stimuli by contraction no longer receives these stimuli and the dilator mechanism, which is still normal, tends to hold the pupils dilated.

In cases of optic atrophy due to syphilis, the pupils may not be maximally dilated, and indeed may be of small size. This may occur in patients who developed Argyll Robertson pupils prior to the onset of optic atrophy. As will be shown later, the sympathetic innervation of the iris is disturbed in the Argyll Robertson pupil. This interferes with the dilator mechanism so that the pupils become small. Possibly the arteries lose their elasticity and the circulation through the iris is disturbed. The pupils remain small even though the sphincter muscle of the iris no longer is under the influence of visual stimuli.

Some time ago we observed a young man with a congenital absence of the cerebellum and apparently with a failure of development of certain structures in the region of the midbrain. This patient had extremely large pupils which were maximally dilated and did not react to any reflex stimulus. Nor did they react to drugs. The sphincter muscle of the iris may never have developed. On the other hand, the dilator mechanism was normally differentiated producing a constant maximal dilatation of the pupils.

THE EFFECT UPON THE PUPIL OF CHANGES IN THE GENERAL CIRCULATION

It would be interesting to make further clinical and experimental observations upon the effect of the disturbances of the general circulation upon the size of the pupil. Vascular changes either local or in the general circulation are of much greater importance than has been realized recently. There may be some correlation between the size of the pupils and the blood pressure. It has been shown that the pupils tend to be constricted by the injection of any substance such as adrenalin which tends to raise the pressure in the general circulation. The changes in size of the pupil produced by raising or lowering the pressure of the injecting fluid have been described. It has also been shown that sectors of the iris may inject more readily, producing a widening of the iris and a flattening of the pupil over a localized portion of the pupillary margin.

Pollack (1914) found that pituitrin applied locally produced a dilation of the normal pupil. When the pituitrin was introduced intravenously the local mydriatic effect was lost and pupillary constriction occurred as the blood pressure rose. As the blood pressure declined, the miosis passed off and was followed by a slight dilatation. Elliott observed the same result after intravenous injection of adrenalin in the dog.

It might be assumed from Pollack's experiments that the constriction of the pupil after the intravenous injection of pituitrin or adrenalin is due to the effect upon the general circulation, raising the blood pressure. When this general effect was finished, the localised effect of the drug upon the iris was manifest.

THE CONCEPT OF THE ARGYLL ROBERTSON PUPIL

Argyll Robertson (1869) described four patients with peculiar abnormalities of the pupils. For the pupils to show the complete picture described by him,

it is necessary that five features be present. One, the retina must be relatively sensitive to light. Two, the pupil must be small. Three, the pupils must remain of constant size, regardless of the strength of the light. Four, they must contract actively on accommodation for near objects. Five, they must dilate imperfectly in response to the instillation of atropine. One additional feature has been suggested. There is no dilatation of the pupils in response to painful stimuli.

The Argyll Robertson pupil is probably the best known pupillary abnormality and is commonly found in syphilis of the central nervous system. The anatomical lesions producing this change have never been satisfactorily demonstrated. Authors have made attempts to locate the position in the brain where an injury would produce the pupillary disturbances. The literature dealing with this subject is enormous.

There has been a tendency for investigators to disregard certain of the five or six criteria in the diagnosis of Argyll Robertson pupils. If the whole picture is present, it may be said that the change is practically pathognomonic of central nervous system syphilis. Adie (1931) stated that he had never seen or found reported in the literature any case satisfying the criteria given by Argyll Robertson in which syphilis of the nervous system was excluded. Naturally, the whole picture is not present in every case of syphilis of the nervous system. Many instances are seen in which there is a partial development of the Argyll Robertson pupil.

Merritt and Moore (1933) summarized the records in 749 cases of neurosyphilis. In dementia paralytica, the pupils were entirely normal in only 5.4 per cent of the cases and in tabes dorsalis in 2.6 per cent. In cerebrovascular syphilis, on the other hand, the pupils were normal in 40.8 per cent.

Wilson (1929) felt that miosis was not essential for diagnosis of the Argyll Robertson pupil. If this point of view is accepted, the phenomenon is not necessarily restricted to syphilis of the nervous system. Argyll Robertson pupils then would occur occasionally in lethargic encephalitis, disseminated sclerosis, tumors of the third ventricle, aqueduct of Sylvius or optic colliculi, syringobulbia, chronic alcoholism or polioencephalitis hemorrhagica superior. Various theories dealing with the anatomical lesions may be considered.

Wilson (1929) advanced the suggestion that toxins were present in the cerebrospinal fluid circulating in the ventricles. These toxins produced subependymal involvement of nervous tissue, spreading from the aqueduct of Sylvius to implicate the afferent fibers responsible for the pupillary light reflexes. The presence of subependymal granulations found commonly in dementia paralytica tended to support this view. However, Argyll Robertson pupils are just as common in tabes dorsalis in which there are rarely any evidences of ependymitis.

Ingvar (1928) thought that basilar meningitis was responsible for the destruction of the fibers in the optic nerves which carry the afferent stimuli for the light reflex. He postulated that the afferent fibers from the retina responsible for the pupillary responses travel at the periphery of the nerve and are therefore particularly vulnerable to attack. The parasympathetic fibers to the eye also

lie on the surface of the oculomotor nerve. Ingvar postulated that if the afferent fibers were destroyed the pupils would no longer react to light and the Argyll Robertson pupil would be established. It is difficult to explain a unilateral Argyll Robertson pupil on this basis. Merritt and Moore pointed out other objections to this hypothesis. The percentage of Argyll Robertson pupils in syphilitic meningitis is very small whereas it is much higher in cases of parenchymatous neurosyphilis. Other forms of chronic basilar meningitis, for example, tuberculous, do not produce Argyll Robertson pupils. Finally the miosis is not adequately explained.

Merritt and Moore believed that the lesion responsible for the Argyll Robertson pupil must lie in some place where it injures not only the afferent visual fibers controlling the light reflex, but also the fibers from the hypothalamic region regulating the sympathetic innervation of the pupils. The latter injury would be responsible for the miosis, failure of the pupils to dilate adequately following the instillation of atropine, and the loss of the response of dilatation to painful stimulation. They placed the lesion ventral to the posterior commissure in the region where the light fibers are coursing ventromedially to the oculomotor nuclei and the pathway from the hypothalamus runs down into the midbrain.

Again there are possible objections to this theory. Merritt and Finland (1930) found that the pupil did not remain miotic after injury of the pathway from the hypothalamus. In the course of weeks or months, the pupil tended to approximate its former size. Moreover there may be some doubt as to whether the failure of reaction to light and preserved reaction to accommodation can be divorced from each other as thoroughly as has usually been done in speaking of Argyll Robertson pupils. Holmes in discussing the tonic pupil suggested that accommodation was a more powerful stimulant of pupillary contraction than light.

If the Argyll Robertson pupil need not be miotic, there are many interesting case reports of abnormal pupils which react not to light but to accommodation dependent upon causes other than syphilis. These may be dependent upon a lesion in the midbrain, severing the visual fibers at the point where they enter the midbrain and make connections with the oculomotor nucleus.

In 1934, the Journal of the American Medical Association published an editorial on the Argyll Robertson pupil, particularly in view of the paper by Merritt and Moore. It was suggested that the pupils were small though not necessarily equal in size because of the intrinsically greater power of the constrictor muscle. Therefore when both constrictor and dilator muscles are paretic, the constrictor action predominates and the pupils are more or less small. Miosis is a frequent though not an essential part of the Argyll Robertson sign. They suggested on logical grounds that the pathway of the accommodation reflex is from cerebral cortex to oculomotor nucleus. The lesion that produces the Argyll Robertson pupil does not affect this pathway and consequently the pupils contract normally on accommodation. They believed that the failure of dilatation of the pupil in response to painful stimuli is due to an injury of the sympathetic reflex arc. In this paper it has been shown that this is not true.

It is agreed at the present time that when the pupils are miotic, answering the complete description of Argyll Robertson, they indicate syphilis of the central nervous system. Of course, the pupil in syphilis may show changes which do not fall under the heading of Argyll Robertson pupil. If we do not insist that the pupil is small, many types of injury can produce an Argyll Robertson pupil.

If a patient is blind, due to atrophy of both optic nerves, the pupils do not react to light, but do react to accommodation. These pupils are usually large. No one suggests that they should be included under the definition of Argyll Robertson pupils. Many types of lesions in the region of the midbrain or diencephalon may produce large pupils reacting to accommodation but not to light. Similarly, it will be shown in an ensuing section that regeneration of a severed oculomotor nerve may give rise to pupils which do not, however, react to light but do react to accommodation. These pupils are not necessarily small. We consider that Argyll Robertson pupils must be miotic.

Too much emphasis cannot be placed upon the actual measurement of the pupillary diameter. Different observers do not agree to a definition of miosis. Adie believed that any pupil was miotic which had a diameter not exceeding two and a half millimeters. Others have chosen a diameter of less than two millimeters as the criterion for a miotic pupil.

From the standpoint of clarity, it would be better to describe accurately the actual anatomical and physiological changes found in the pupil in cases of neurosyphilis and disregard the label of Argyll Robertson pupil which has different connotations to different people. Thus in any description mention should be made of the exact diameter of the pupil, the equality or inequality of the two pupils and the contour of the pupillary margins. It is then important to record the measured reactions of the pupils to light, accommodation, painful stimuli, forceful closure of the eyes and to drugs. In this way, exact information is recorded which would serve as a basis for more accurate clinical conclusions and be valuable for investigative work.

OBJECTIONS TO THEORIES WHICH POSTULATE A LESION IN THE CENTRAL NERVOUS SYSTEM

It is usually assumed that the lesion responsible for the Argyll Robertson pupil lies in the central nervous system at the level of the upper portion of the midbrain, interrupting the optic reflex fibers which connect with the oculomotor nuclei. Any theory must explain the fact that Argyll Robertson pupils occasionally occur unilaterally. Merritt and Moore suggested that the lesion lies just lateral to the aqueduct of Sylvius injuring both the uncrossed and the crossed optic reflex fibers to the oculomotor nucleus.

An objection to this hypothesis immediately appears. It is known that the fibers of the oculomotor nucleus to the pupils are both crossed and uncrossed, so that one nucleus affects the activity of both pupils at the same time. Under these circumstances, in order to have a unilateral Argyll Robertson pupil, it would be necessary to have an injury on the motor side of the reflex arc peripheral to the oculomotor nuclei.

Merritt and Moore realized that any adequate explanation of the Argyll Robertson pupil must take into account not only that the reflex pathway for the light reflex was injured but that also there was an involvement of the sympathetic innervation of the pupil. The small size of the pupil suggests some injury of the sympathetic innervation. Often in patients with syphilis, there is a ptosis of the lids, again suggesting damage to the sympathetic innervation of the eye. Merritt and Moore postulated therefore that the Argyll Robertson pupil injured not only the optic reflex fibers to the oculomotor nucleus but also the pathway from the center in the hypothalamus controlling the sympathetic activity of the pupil itself. It is known, however, that after an injury of the central pathway controlling the sympathetic fibers there is a considerable return of function in the course of time. The most severe injuries to the sympathetic supply of the iris are those which occur peripherally. It would therefore be more logical to believe that the motor sympathetic and parasympathetic fibers are injured peripherally rather than in the central nervous system.

There is another abnormality of the iris often found in syphilis which is not described in the paper by Argyll Robertson. The pupil is usually irregular in shape. Under the experimental findings a method has been described by which this irregularity in shape of the pupil could be produced. Thus a greater dilatation of the blood vessels in sectors of the iris cause an expansion of the iris locally and a flattening of the pupil upon that side, producing an irregularity in the normal circular shape of the pupil. It is probable that this is usually the mechanism of pupillary irregularity and may be dependent upon faulty nervous control of the blood vessels to sectors of the iris. This would again suggest that the lesion is peripheral. The damage to the pathway from the hypothalamus would affect the blood vessels in all portions of the iris, but not in local sectors.

In summary, therefore, a unilateral Argyll Robertson pupil would be explained only by injury of the motor side of the parasympathetic reflex are inasmuch as one oculomotor nucleus sends fibers to both pupils. The irregularity in shape of the pupils which is commonly found in syphilis of the nervous system suggests a peripheral lesion of the sympathetic innervation. Merritt and Moore suggested that both sympathetic and parasympathetic functions of the iris must be injured at the same time in order to produce an Argyll Robertson pupil. This is undoubtedly true.

LOSS OF LIGHT REFLEX AND PRESERVATION OF ACCOMMODATION ASSOCIATED WITH REGENERATION OF THE OCULOMOTOR NERVE

In several places in the literature it is suggested that damage to the oculomotor nerve may give rise to a pupil which reacts to accommodation but not to light. Bender and Fulton (1938) observed the regeneration of the third nerve in the chimpanzee. Ford, Walsh and King (1941) made a study of the motor disorders following regenerating of the oculomotor nerve in man. These included pupillary changes which were described as Argyll Robertson pupils. However, the pupil was not necessarily small, although in some cases it was of smaller size than the normal. The authors showed that these phenomena were most easily

explained on the basis of misdirection of regenerating nerve fibers which became widely distributed among muscles other than those which they originally innervated.

To consider for a moment muscles other than those of the pupil supplied by the oculomotor nerve, it was found that after regeneration of the nerve there was a loss of elevation and depression of the eye. Instead, there was adduction of the bulb when elevation or depression were attempted. Adduction and abduction of the eye were preserved. During adduction, there was a lifting of the eyelid which also occurred during convergence and attempted elevation and depression. When the eye was abducted, the eyelid drooped. These anomalies were the result of misdirection of regenerating nerve fibers which stimulated muscles other than those which they originally supplied. Since there is a great multiplication of the fibers during the process of regeneration, it is theoretically possible for them to become so widely dispersed that a few of those which formerly supplied any given muscle may grow into every muscle which the third nerve supplies. Consequently, when any movement is attempted in which the muscles supplied by the third nerve play an active role, all the muscles contract simultaneously. As a result of this mass contraction the bulb is adducted for the contraction of the internal rectus is not opposed by the unaffected external rectus. The simultaneous contraction of the elevators and depressors prevents any movement in the vertical plane. The levator palpebrae also contracts causing a lifting of the upper lid. When the patient attempts to elevate or depress the eye, the bulb is adducted and the lid lifts, though no movement in the vertical plane results.

Adduction may be performed as part of the act of convergence or of lateral conjugate deviation and the lid also lifts at the same time. No matter what is attempted, the bulb is adducted and the lid lifts. The loss of movement in the vertical plane is due to simultaneous contraction of the elevators and depressors.

It is not a result of paralysis. Abduction, a movement which does not demand any active participation of the third nerve muscles, is well performed. During this movement, the lid droops perceptibly. During abduction, the tonus of the internal rectus is inhibited and the superior and inferior recti may be partially inhibited since they have some action as adductors. Since the levator palpebrae has received some of the original nerve supply of these muscles, it might be expected to relax during abduction.

Ford, Walsh and King found three types of reaction in the pupil after the oculomotor nerve had regenerated. One, the pupil sometimes remained dilated and fixed, if no regenerating axones succeeded in forming effective synapses in the ciliary ganglion. Two, the pupil might show no light reflex but contract during convergence and also during other movements, such as adduction, which are not normally associated with pupillary contraction. Three, the pupil may contract and show a light reflex, although the latter is slow and of small amplitude.

These authors found that the pupil might contract not only during convergence but when other movements were attempted which required the action of the muscles innervated by the third nerve. When the eye was abducted the pupil

dilated. The absence of the light reflex may be explained by the assumption that none of the fibers normally concerned with the light reflex formed effective connections with the ciliary ganglion. The fact that the pupil contracted not only during convergence but during other movements of the bulb which are not normally associated with the contraction of the pupil would seem to require the assumption that the fibers formerly distributed to the extraocular muscles strayed into the ciliary ganglion and formed synapses with the postganglionic neurones. The total number of these fibers is probably much greater than those normally concerned with the light reflex. They are more apt to reach the ciliary ganglion. The dilatation of the pupil during abduction may be due to inhibition of the nerve fibers which formerly supplied the internal rectus, since the tonus of this muscle is inhibited during abduction.

In two cases they found the pupil of the affected eye to be somewhat smaller than that of the normal eye. This fact seemed to indicate that the constrictor of the pupil was receiving an excess of stimuli. No doubt the pupils are now influenced by many of the stimuli which originally traveled to the various extraocular muscles. Hence the tonic contraction resulted.

CHANGES REPORTED IN THE IRIS IN SYPHILIS

Several workers have described local changes in the ciliary ganglion or iris in cases of syphilis with Argyll Robertson pupils. Marina (1901) believed that he could demonstrate degeneration in the ciliary ganglion which would explain the changes in localized areas of the iris during the period when the Argyll Robertson pupil is developing. It is known that stimulation of short ciliary groups of parasympathetic fibers to the iris may produce localized contraction of the pupil and similarly that excitation of one of the long ciliary nerves may elicit a partial dilatation. Marina suggested that myosis was due to an earlier involvement of the dilator sympathetic fibers, whereas the occasional dilatation of the pupil suggested a primary damage to the parasympathetic constrictor fibers. The inequality of the pupils was explained by the difference in intensity of the degeneration of the ciliary ganglion on the two sides. Atrophy of the stroma, due to nerve injury, gradually produces an inert membrane which is not only unresponsive to reflex stimuli, but also responds poorly to drugs.

Dupuy-Dutemps (1905) observed in a large number of cases where the Argyll Robertson pupil existed, a peculiar atrophy of the iris which could be observed without complicated apparatus by simply looking at the eye. He felt that the radial folds which are usually found in the mesial portion of the iris were lost and the crypts which normally occur more laterally tended to disappear. The surface of the iris under these conditions was flat, showing only a fine striation. In the region of the pupil the iris, which is normally thick, became thin, and there was a change in the distribution and appearance of the pigment.

Between the ultimate state of atrophy of the iris and the normal there were all intermediate forms. He felt that the changes were more marked in the medial portion of the iris than in the ciliary portion. It was not rare to observe a partial atrophy which involved a sector of the iris and predominated there, while the

remainder of the membrane was little modified. This formed a contrast between the atrophic and the normal portion. The extent of the involved sector was variable and two or more atrophic sectors might be separated by intervals of normal tissue. These changes did not involve predominantly any particular portion of the iris.

Dupuy-Dutemps felt that these changes were quite different from the extreme degree of atrophy which is present in cases of long and severe inflammation where the iris is profoundly disorganized. In old people, the iris often shows a senile involution so that the iris is thinner and shows fewer folds than in the normal adult. Myopic eyes show this to a marked degree; the folds are smoothed out and there is a general atrophy of the uveal tract. These changes are not the same as those found in the case of the Argyll Robertson pupil.

If the atrophy of the iris is partial and occurs in sectors, it produces a change in the shape of the pupil. When the iris is uniformly altered, the pupil will be fairly circular. Irregularity in the pupillary margins is related then to the unequal atrophy in different portions of the iris.

In following the development of the Argyll Robertson pupil, the author pointed out that the response to pain is lost early, at the time when the light reflex is less adequate than normal. The contraction of the pupil on an attempt to close the eyelids forcibly is lost later, at a time when there is a loss of response to accommodation. The diminution in response to light is often lost before there is any appreciable atrophy of the iris. The alterations of the iris which can be seen with the eye may become manifest about three years after the first sign of diminution in the light reflex. Then, on observation, it will be found that a localized atrophic zone of the iris is absolutely immobile to light while the rest of the iris may show a fairly normal reaction, although it is slow. This is a partial Argyll Robertson pupil. Its significance is important from the point of view of explanation of the physiology. Under these conditions, accommodation occurs with about the same intensity in all parts of the pupil. When the atrophy occurs predominantly in one sector of the iris, it is sometimes possible to observe enfeeblement or abolition of the accommodation reflex in the altered zone while it persists in other portions of the pupil. Loss of the reflex contraction of the pupil on an attempt to close the eyes appears only when the atrophy of the iris is marked.

The author concluded from observation that injury of the oculomotor nerve proximal to the ciliary ganglion never produces an atrophy of the iris. With a lesion of the oculomotor nerve, the pupil would be large. He also believed that the atrophy of the iris is independent of injury of the cervical sympathetic trunk. In the case in which he studied the changes of the iris histologically, he found simple atrophy without a trace of inflammatory change. The connective tissue of the stroma was diminished, the muscular fibers of the sphincter were less numerous and arranged in thin bands. There existed in certain portions large deposits of abnormal pigment.

Dupuy-Dutemps suggested two possible explanations for the Argyll Robertson pupil. In the first place, there might be a toxic or infective lesion involving

the iris tissue directly. This theory was discarded because acute or chronic inflammation of the pupil does not produce an Argyll Robertson phenomenon. Segmental changes in the iris are incompatible with a diffuse type of lesion. It seemed more reasonable to the author that the change in the iris was secondary to involvement of the short ciliary nerves or the cells of the ciliary ganglion. He thought that injury of the postganglionic parasympathetic neurones was alone capable of producing the atrophic alterations in the iris which he described. He pointed out that the sensory fibers reaching the iris have their cells of origin in the ganglion of the trigeminal nerve. In people with Argyll Robertson pupils the corneal reflex is not abolished, showing that many of the sensory fibers of the trigeminal are not injured.

McGrath (1932) emphasized that in addition to the ptosis and pupillary abnormalities, there are equally constant changes in the appearance of the iris in neurosyphilis which are not commonly recognized. They include differences in the color and irregular pigment deposition and abnormalities in the texture of the iris stroma. The second of these two changes is most readily seen in blue eyed subjects in whom the stroma is not concealed by pigment as in dark eyed persons.

The author described a typical case of Argyll Robertson pupil in a woman of 50. Both pupils were small, of 2 millimeter diameter, irregularly oval, completely inactive to light but reacting to accommodation. All the other typical signs of tabes were present. The irides were a pale, discolored blue. The radial strands of the stroma were abnormally thin and had almost lost their normal sinuosity, looking as though they had been combed out straight. The circulus minor and the iris crypts had disappeared. The pupillary aperture was irregularly oval and of a different form in each eye. The posterior pigmented layer was irregularly visible and was absent at certain points in the margin of the iris. The author claimed that this type of change is invariably found in neurosyphilis and never apart from it.

Changes in iris color and texture may develop at first in one or more segments of the iris and there attain full development while the rest of the iris remains normal. The pupillary outline is altered by these segmental changes in the iris and it is at the point where the altered segment impinges upon the pupillary margin that deviations from the circular outline of the pupil first appear and it is here that the first signs of defect in response to light are seen. The light reaction is defective or lost in pupillary segments adjacent to altered iris sectors

McGrath gave an example of a woman of 42 with asymptomatic neurosyphilis. The pupil of the left eye was three millimeters in diameter and irregular in outline. The iris in two separate sectors was of a pale, watery blue; the rest being of a darker tint. In these two sectors, the circulus minor and the iris crypts were lost. The radial stroma fibers were notably thin and lost much of their sinuous outline. In the two segments of the pupil on which these segments impinged, the pupil deviated from the circular outline and the posterior pigmented layer was narrower than elsewhere. The pupil contracted with normal speed to light except in the two affected segments where no light reaction whatsoever occurred.

Another example of segmental degeneration of the iris was found in a woman of 54 with taboparesis. In the left eye, the nasal sector of the iris was atrophic and paler than the temporal sector. In the nasal half, the circulus minor was distorted and indistinct. There was miosis and the pupil was pulled out of the circular in the nasal half. The nasal sector responded neither to light nor upon convergence, but in the temporal half a sluggish reaction to convergence remained. When examined 15 minutes after death, the pupil was found widely dilated, but the irregularity of outline was accentuated. The atrophic half of the retina had retracted more than the normal half.

This segmental atrophy of the iris is found in association with incomplete loss of the light reaction. It may be regarded as representing a stage in the evolution of the complete Argyll Robertson pupil as it has been defined. Light reaction when studied in a series of cases in which at first it is incompletely lost is found to diminish and then to disappear, not by gradual uniform failure all around the pupil but locally in one or more segments. Until it is completely lost, a careful examination of the pupil with the eye shaded shows that on illumination the pupil reacts irregularly, the response being absent at one part of the pupillary margin, feeble in another and still fairly active in yet others. In many tabetic eyes there is a patchy deposition of pigment in the form of fine dustlike granules in the more atrophic sectors of the iris. This may represent the initial change in the process which is described here as iris atrophy which includes thinning and straightening of the iris stroma, fading and discoloration of the iris, and irregularity of pupillary outline.

Fifteen cases of miotic Argyll Robertson pupil were observed immediately after death and in all wide dilatation of the pupils which remained irregular in outline, was observed to occur in fifteen minutes.

In the normal eye, the instillation of 0.5 to 1 per cent solution of atropine causes maximum dilatation after about 30 to 45 minutes and complete paralysis of the convergence reflex occurs. In cases of complete Argyll Robertson pupil, the orifice dilated to possibly twice its original diameter after three hours. Any existing irregularities of pupillary outline were accentuated after this imperfect dilatation. The effect of the drug did not pass off for six to twelve days. In no case was there complete failure of the pupil to dilate.

The normal pupil begins to constrict and show spasms of accommodation within five minutes after the instillation of eserine and a maximal effect can be seen within an hour. The effect persists for some twelve hours. In the case of the Argyll Robertson pupil the maximal constriction takes approximately two hours to show itself. A previously dilated pupil, when contracted, shows clearly the presence of atrophic changes in the iris.

In ten cases of complete Argyll Robertson pupil, homatropine caused a slow medium dilatation after two and a half hours, the increased diameter of the pupil being only two to three millimeters. Cocaine was then instilled in the eyes which were previously dilated with homatropine but no further dilatation of the pupils resulted. In six cases of complete Argyll Robertson pupil, cocaine was injected with the following results: in five, the pupillary diameter increased by

only one millimeter after two and a half hours, while in the other eleven cocaine caused no dilatation of the pupils after three hours.

The author adopted two millimeters diameter as a standard for miosis of the pupil. He found miosis in all cases of tabes, but in only four per cent of the cases of paresis. In paresis, the average pupillary diameter was four millimeters. In paresis, fifty per cent of the cases showed unequal pupils; in tabetics, sixty per cent; seventy per cent of taboparesis showed inequality of the pupils. Irregularity of pupillary outline was present in almost all cases of taboparesis, but only in thirty-six per cent of the cases of paresis.

McGrath then discussed the theories of the development of the Argyll Robertson pupil. The one most generally accepted suggests that lesions are present in the gray matter around the aqueduct and the mid-brain where they interrupt the optic connections to the third nerve nucleus subserving the light reflex. This was Kinnier Wilson's hypothesis. He regarded miosis as an incidental phenomenon for which a separate hypothesis was required. This theory gave no adequate explanation for the irregularity of the pupillary outline or for pupillary inequality. The existence of changes in the structure of the iris was not recognized. His theory owes its existence to a frank disregard for all but a single component of the Argyll Robertson pupil, that is, the loss of direct light reflex.

McGrath presented five reasons why this hypothesis cannot be accepted. One, it does not account for trophic changes in the iris. Two, it does not explain the diminution and later loss of the light reaction locally in the iris in close correspondence with these trophic changes. Three, it does not explain the irregularity of pupillary outline or the miosis, both of which are essential components of the Argyll Robertson pupillary syndrome. Four, it fails to account for the defects in response of the Argyll Robertson pupil to drugs, such as cocaine, atropine and eserine. Five, it does not explain the occasional occurrence of unilateral Argyll Robertson pupil. It is known that each third nerve nucleus sends fibers to both ciliary ganglia. Therefore, bilateral loss of the light reflex is inevitable if the underlying lesion be central. The author believed that the injury was in the ciliary ganglion or optic bulb.

The so-called Argyll Robertson pupil of mesencephalic tumors or certain nonsyphilitic diseases, or, as it is sometimes called, the pseudo-Argyll Robertson pupil, is somewhat different than the true Argyll Robertson pupil. The affected pupil is dilated and circular and the iris presents none of the abnormalities that have been described.

Lowry (1941) described a simple method of examining the iris to determine syphilitic changes. He instructed the patient to look the examiner in the eye and projected a light from a small pocket flashlamp across the anterior surface of the iris from the temporal side to the nasal side. This procedure throws depressions into shadow and illuminates raised areas.

He stated that the irides of syphilitic patients present several deviations from the normal. One, there are small ovoid depressions in the superficial mesoblastic layer which are similar to the crypts in appearance but are not confined to the border of the ciliary zone; they are also seen in the central ciliary portion, and are

more numerous than usual. Two, the circulus iridis minor is in many cases not concentric with the pupillary margin of the iris, but is eccentric. It has the appearance of having been pulled to one side away from the pupil. Three, the angle of Fuchs between the pupillary and ciliary portions of the iris is increased and the pupillary segment appears in consequence to be set on a plane posterior to that of the ciliary zone.

Lowry examined 1,769 patients. He believed that the changes which he has described may be present as early as the secondary stage of the disease. He recognized syphilis by this method in 86.2 per cent of the cases, with a fifteen per cent error. It was the author's impression that the changes are due to a cellular infiltration around the blood vessels of the iris during the early stages of the disease and that following the regression of this inflammatory change, scar tissue is formed which produces the crypts and an irregularity of the circulus iridis minor. In sections he has been able to demonstrate an increase in plasma cells in the iris.

POSSIBLE EXPLANATION FOR ARGYLL ROBERTSON PUPIL AND SYPHILITIC CHANGES IN THE PUPIL ON THE BASIS OF A PERIPHERAL LESION

Certain evidence has been presented that the Argyll Robertson pupil is dependent not upon a lesion in the central nervous system, but upon changes in the peripheral nerves either in the orbit or in the iris itself. The irregularity of pupillary outline and abnormalities in the structures of the iris suggest a peripheral lesion. The possibility of unilateral changes could be explained on this basis. There is no positive evidence of damage in the central nervous system which produces the changes in the pupils found in neurosyphilis.

In tabes and syphilitic optic atrophy, also, there is no reasonable explanation for the way in which the nerves are damaged. Few spirochetes can be demonstrated in the injured area. There is a growing tendency to think that tabes dorsalis is dependent upon occlusion of the small blood vessels supplying the dorsal portion of the spinal cord and the posterior roots. Patients usually live for a considerable period after the syphilitic lesions have become marked and it is difficult by histological study to retrace the steps in their development.

Three groups of fibers entering into the innervation of the iris have been demonstrated; sympathetic, parasympathetic, and sensory fibers. It is possible to speculate as to what fibers would be involved in the periphery in order to produce iris changes similar to those found in syphilis.

The sympathetic fibers supply the blood vessels, the pigment cells and the stroma of the iris. There is considerable evidence that in syphilis, this group of fibers is thrown out of function in the production of the abnormalities in the pupil. Thus, there is often, in addition to the pupillary changes, a ptosis of the eyelids of the type seen with paralysis of the sympathetic fibers. The pupil is small and in advanced stages is even smaller than that commonly found with a pure injury of the sympathetic fibers to the iris. Changes in the arrangement of the pigment in the iris have been described. A certain thinning of the iris has been mentioned in certain cases, the blood vessels seem to be straightened out, and the folds in the iris become less prominent. All these things could be explained by a lesion of the sympathetic fibers.

The possibility of an involvement of the sensory fibers to the iris reaching the iris through the trigeminal nerve may next be considered. These sensory fibers have been noted to end among the fibers of the sphincter muscle of the pupil and also along the course of blood vessels near the pupillary margin. It is assumed that the endings in the smooth muscle are stretch receptors, having to do with the tone and contraction of the sphincter muscle. In cases of tabes dorsalis, the symptoms are due in large part to an injury of proprioceptive fibers to muscles and tendons. The patients are ataxic since they can no longer recognize the position of the muscles and they become insensitive to deep pressure pain, vibratory sense and passive movement. Moreover, it has been shown that the bladder symptoms in tabes are largely the result of injury to the sensory proprioceptive fibers ending on the smooth muscle in the bladder wall. The tone of the vesical muscle is decreased and the sense of pain on muscle stretching is lost. The patients no longer have a desire to empty the bladder.

The injury of proprioceptive fibers in both striated and smooth muscle, therefore, is a characteristic of tabes. This produces a diminution of tone in the muscle and secondary difficulties in muscle contraction. The diminution in response of the iris sphincter evidenced by a loss of response to light and a diminution of response to accommodation, as well as a lack of adaptation to other stimuli such as pain and drugs may be dependent upon loss of proprioceptive fibers from the sphincter muscle. This is a speculation which it would be difficult to verify since it would be almost impossible to obtain histological proof.

One argument has been raised against this hypothesis. The corneal reflex is scarcely diminished in patients with syphilis, so that pain fibers from the cornea still reach the central nervous system. However, in the case of tabes there is a selective involvement of sensory fibers. Other types of sensation are often relatively well preserved after the proprioceptive fibers are injured, so that this would not necessarily be an argument against the hypothesis.

Finally, the possibility of injury of the parasympathetic fibers to the iris must be considered. The injury must be on the effector side of the reflex arc in order to account for a unilateral Argyll Robertson pupil. The autonomic portion of the third nerve nucleus innervating the pupil sends fibers to both irides. If accommodation is a more powerful stimulus to pupillary contraction than is light, then the relative preservation of the accommodation reflex, related to the complete loss of the response to light, could be understood.

The report by Ford, Walsh and King (1941), which has been already summarized in detail, explains the method by which regeneration of the oculomotor nerve may produce changes in the pupil somewhat similar to those found in syphilis. Thus in certain cases there is a reaction to accommodation but not to light and sometimes the pupil is smaller than the one upon the opposite side. The Argyll Robertson pupil in syphilis might be due to a peripheral lesion in the oculomotor nerve, producing the changes which were described in that paper. However, there is no apparent disturbance of contraction of the striated muscle innervated by the oculomotor nerve such as occurs when this nerve regenerates.

It would seem likely that the involvement of the sympathetic and parasympathetic fibers occurs in the same region, close to the bulb or in the eye itself and

that any lesion of the parasympathetic fibers affects the cells of the ciliary ganglia or the short ciliary nerves. Certainly, most facts point to an involvement of the iris itself, damaging the sympathetic, parasympathetic and probably the sensory fibers. Syphilis has a tendency to involve blood vessels and this may be the primary cause of the nerve changes. The fact that the pupil is often smaller than would be accounted for by loss of its sympathetic fibers suggests primary atrophy of the blood vessels and other structures in the stroma.

Merritt and Moore realized that the small size of the pupils cannot be due to any increased tone in the sphincter muscle. Indeed, everything points to the fact that if anything, the sphincter muscle tends to lose its tone and power of contraction. It is probable that the sphincter muscle of the iris is at a point of rest when the pupil is small. Normally the pupil is dilated in a blind eye. The constrictor muscle in syphilitic pupils has lost its power of reaction to pain stimuli which normally produces reflex dilatation. Moreover the dilator recoil is absent, hampering sphincter action. The sympathetic control is lost and the blood vessels appear to have lost their inherent elastic property.

With a paralysis of the sympathetic fibers to the pupil, even a normal sphincter muscle does not act as efficiently in dilatation or contraction as it could in a normal eye. Although the pupil still contracts and dilates, its movements are somewhat limited. The weakness of the dilator mechanism appears to be more marked in the Argyll Robertson pupil than after section of the sympathetic fibers. This in itself would indicate that the lesion was peripheral involving not only the sympathetic fibers, but possibly the walls of the blood vessels.

Atropine exerts its effect upon the iris by paralysing the sphincter muscle, producing a dilatation of the pupil. This dilatation is much less complete after the sympathetic fibers to the pupil have been cut. This would show that dilatation of the pupil depends not only upon the relaxation of the sphincter muscle, but also upon the active pull of the dilator mechanism. The fact that atropine acts poorly upon the Argyll Robertson pupil shows in itself that injury of the sympathetic fibers to the iris is present.

The Argyll Robertson pupil dilates well at death but retains its irregular shape suggesting local changes in the structures of the iris. This dilatation may be in large part dependent upon the drainage of blood from the iris vessels. The relaxation of the sphincter might suggest that during life it is in active contraction.

Inasmuch as tabes dorsalis, Argyll Robertson pupils and primary optic atrophy occur so frequently in the same individual, it might be assumed that the process which produces these symptoms has certain features in common. In none of the three is the process understood. Tabes dorsalis is a condition affecting the nerve roots close to the spinal cord. There are usually no primary changes in the cord itself. Primary optic atrophy is dependent on changes outside of the brain. The pupillary changes may have a peripheral origin.

THE TONIC PUPIL

The peculiar phenomenon of the tonic pupil was first described independently by Strasburger and Saenger in Germany in 1902 and became well known following

the description of Adie in 1931 and 1932. Many opthalmologists and neurologists have written upon the subject (Markus, 1906; Moore, 1924, 1925 and 1932; Parks-Weber, 1923 and Weil and Reys, 1926).

This abnormality occurs in otherwise healthy individuals in whom there is no history of syphilis and the serological test for syphilis is negative in the blood and spinal fluid. A great majority of the patients are women. In many cases the knee and ankle jerks are lost or very sluggish. The left pupil has been involved more often than the right in 54 cases reported (Moore, 1932). Occasionally both pupils are affected.

Holmes (1932) defined the tonic pupil as follows: "It is characterized by loss of the pupillary reflex to light, often associated with disturbances in the reaction to convergence, though this is usually better preserved, probably owing to the effort of convergence being a more potent stimulus, while other reactions of the pupil, such as to pain or sympathetic stimulation and the contraction that occurs normally on firm closure of the eyelid may also be preserved." In six cases the pupils were somewhat irregular and in two they were eccentric. They reacted normally to atropine and eserine. Some of Holmes' patients complained of mild pains or paresthesias in the extremities at the onset of the pupillary disturbances. One stated that for a time her gait was unsteady or ataxic and a few suffered with mild headaches, possibly owing to sudden dilatation of the pupil or disturbance of accommodation. In 19, there were definite pathological changes in the reflexes. Holmes believed that these patients suffered from diffuse or widespread involvement of the central nervous system, of which the pupillary phenomena formed only a part. The symptoms suggested that the disease might be of the nature of an infection to which the reflex mechanism of the pupils is particularly vulnerable.

Jelliffe (1933) in a critical review confirmed Adie's belief that myotonic pupils are common and reported two cases associated with hypothyroidism. Whether the syndrome is a disease, as claimed by Adie, is doubtful. The changes in the pupils and in the deep reflexes may be associated with alcoholism, influenza, encephalitis, diabetes, alcoholic polyneuritis, and hypothyroid states.

It is difficult to date the onset of the pupillary changes in many cases but the patient or friends may notice that one pupil is larger than the other. The patient himself may observe a blurring of vision when he attempts to read, due to the difficulty in adjustment of the pupils for accommodation. The inequality may first be noticed when the individual comes to the hospital for another reason. The onset has been observed in many cases between the ages of 20 and 30 years. The condition has been observed in siblings (Moore, 1925) and in twins. Probably the tonic pupil may be present at birth.

The affected pupil is usually somewhat larger than the normal. It changes in size and may become equal to or smaller than the normal one. There is no immediate response to light. The pupil reacts, although slowly and imperfectly to accommodation. If the patient is placed in a dark room, the pupil does not dilate at once. After a considerable period, a half hour or more, the pupil dilates, and may become larger than the normal. Then on return to light, the pupil

slowly contracts. It may first become smaller than normal and finally dilates to the usual size.

Painful stimulation of the skin of the neck produces a normal dilatation of the pupil. The pupil also dilates on forceful closure of the lid and responds normally to drugs such as atropine and eserine. The visual acuity is normal.

Holmes (1932) observed nineteen cases, all of them women. There were other signs of involvement of the nervous system in each one. These had to do chiefly with disturbances of the reflexes. Ten of the nineteen cases were between the ages of twenty-one and twenty-five years. Only five were over thirty years. There was in some cases a progressive loss of reflexes while the patient was under observation. In fifteen, the pupillary changes were unilateral and in four bilateral. Three stated that the pupils were affected in succession. One patient noticed that the pupil was usually large but became pin point in size when she tired. Another observed that the large pupil became smaller than the normal one when she wept. One case reported spasms of accommodation and in all the patients, accommodation had a certain tonic quality.

The condition developed rapidly in most cases and the pupil remained abnormal. One tonic pupil has been known to have been present for forty years. In a small proportion the abnormality spreads to the other eye.

Because of the association of abnormal pupils with absent tendon reflexes, a diagnosis of syphilis must always be considered. The pupil differs, of course, from an Argyll Robertson pupil, in that it is not miotic. Adie pointed out that the reaction to light is not lost, but masked by the undue tonicity of the pupil.

There is no information concerning the anatomical changes in these cases and none of the speculations are satisfactory. Most observers feel that the abnormality is not in the muscle itself, and that it cannot be thought of as myotonia, similar to Thomsen's disease. Holmes noticed that some of the patients complained of mild paresis or paresthesia in the extremities at the time of the onset of the pupillary disturbances. He thought the disease was of the nature of an infection which involved the third nerve or its nucleus in the brain stem and also produced changes in the spinal cord. The manner in which the lesion of the third nerve nucleus would give rise to the symptoms was not explained. German authors have suggested that the lesions were supranuclear producing an increase in tone in the muscle having to do with pupillary changes, the sphincter muscle of the eye. Inman (1925) believed that emotional stress would bring on the condition. Morgan and Symonds (1927 and 1931) considered the tonic pupil a manifestation of encephalitis.

THEORETICAL INTERPRETATION OF TONIC PUPILS

It is even more difficult to speculate concerning the cause of tonic pupils than of Argyll Robertson pupils. No evidence has ever been produced that the slowness of these pupils in contraction is due to any disease of the muscle itself such as the myotonia which is sometimes found in striated muscle. Moreover,

there are no data which suggest that the lesion is in the supranuclear structures, controlling the oculomotor nucleus. Indeed, no cases have been studied from a pathological point of view.

Holmes pointed out the interesting fact that many cases of tonic pupil had symptoms suggesting a mild polyneuritis. Moreover, a number of the patients show an absence or diminution of the ankle jerks and knee jerks which would fit in with a possible polyneuritis. Holmes himself thought that the lesion might be in the oculomotor nucleus or in the third nerve.

Ford, Walsh and King have described the types of abnormalities in the pupils found after regeneration of the third nerve, but they are quite different from those found in tonic pupils. The pupils reacted to accommodation and not to light, but their response was prompt when a suitable stimulus was used. It seems difficult to explain the findings on the basis of a lesion of the oculomotor nerve. There are no findings to suggest that the sympathetic innervation of the pupil is involved in any way. No change in the pigment of the iris or in the structures of the stroma have been described. The pupils react well to drugs.

In six of the 19 cases described by Holmes and in the reports of others the pupils were irregular in outline. This must be due to different degrees of change in different sectors of the iris. The damage must be selective in the involvement of the nerve fibers to the iris sectors. This almost requires the injury of nerve fibers in the periphery.

There is no direct evidence of involvement of sympathetic or parasympathetic fibers to the iris. This leaves but the possibility of involvement of the sensory fibers, the third peripheral group which innervate the iris. These sensory fibers may be considered to supply stretch receptors to the sphincter muscle of the iris. In smooth muscle as in striated muscle, it is probable that the tone of the muscle fibers is dependent primarily upon sensory stimuli from muscle itself. These sensory stimuli of a proprioceptive nature therefore, are extremely important in the normal functioning of the muscle. If it is true that the patient with tonic pupils has a peripheral neuritis, this might involve the proprioceptive sensory fibers from the pupil leaving the other fibers intact. Under these conditions, the pupil would react slowly and abnormally to any type of stimulus.

In order to test out this hypothesis, it would be interesting to study the reactions of the pupil after the ophthalamic branch of the trigeminal nerve was sectioned, cutting the proprioceptive fibers from the iris. Possibly data of this type could be obtained which would be helpful in defining the function of the proprioceptive fibers from the constrictor muscle.

Reaction to accommodation is better preserved than responses to light both in the Argyll Robertson and tonic pupils. The light reflex continually plays on the iris whereas the accommodation reflex is more infrequently evoked and has a certain voluntary component. As shown by the great dilatation of the pupil in blind eyes which equals the dilatation after section of the parasympathetic fibers it may be said that light is the most potent stimulus to pupillary contrac-

tion. In explaining their dissociation an analogy with the loss of movement in the Parkinsonian syndrome might be considered. There the fleeting changes of facial expression and associated reflexes such as swinging the arms in walking are most severely involved. These require little effort or attention in normal individuals. The voluntary movements are less severely affected.

SUMMARY

The description given by Argyll Robertson himself for the abnormality of the pupil known by his name has been accepted in this paper. Thus, the Argyll Robertson pupil is a small pupil which fails to react to light but reacts on accommodation. The smallness of the pupil is often disregarded as a criterion. Many types of lesions in the central and peripheral nervous system may produce a large pupil which fails to react to light, but reacts on accommodation. This occurs with injury of the optic nerve and with regeneration of the oculomotor nerve. It can undoubtedly be produced by lesions in the region of the diencephalon or midbrain; for example, by tumors in the pineal region. Fundamentally, it would be more constructive to discuss not the Argyll Robertson pupil but the pupillary abnormalities related to syphilis of the nervous system. This includes irregularity of the pupillary margin which was not described by Argyll Robertson.

Although the majority of neurologists ascribe the Argyll Robertson pupil to a lesion at the upper end of the midbrain, there is no pathological or clinical evidence that this is the point of injury. The fact that the pupil is small must be ascribed in part, at least, to a lesion of the sympathetic fibers. Merritt and Moore escaped this difficulty by assuming that there is an injury of the fibers from the hypothalamus controlling the sympathetic innervation of the iris in this same region in the midbrain where fibers of the optic nerve making connection with the oculomotor nucleus are damaged. The type of abnormality described by Merritt and Moore would not explain a unilateral Argyll Robertson pupil. The oculomotor nucleus innervates the iris of both sides so that the injury must be on the motor side of the reflex arc. Moreover, an injury in the central nervous system would not account for the irregularity of the pupil which is so often present. This irregularity is due to the fact that portions of the iris contract or expand where other portions are less responsive to reflex stimuli. Variations in width of different portions of the iris may be due to a greater injection of the blood vessels in some sectors of the iris than in others.

A peripheral injury in the region of the iris would explain the abnormalities of the pupil in a more satisfactory way than a lesion of the central nervous system. In the former case, the damage could be unilateral producing an Argyll Robertson pupil upon one side. Local injury of the sympathetic fibers controlling the blood vessels of sectors of the iris would cause an inequality of the pupil during the development of a complete miotic Argyll Robertson pupil.

Certain workers have described structural changes in the iris in cases of

syphilis producing abnormalities of the pupils. As these develop, they are said to occur first in localized sectors of the iris. There is a change in the distribution of pigment and a general atrophy of structures in the stroma, probably including the blood vessels. Certain portions of the iris remain reactive while other sectors no longer react to light or drugs. While these findings are not supported by a great number of observations, they offer a field for further study which may be a very fruitful one. It is our belief that the lesions producing changes in the iris in syphilis are peripheral in the region of the iris itself, damaging the sympathetic, parasympathetic and sensory fibers to the pupil.

Possibly an injury of the proprioceptive fibers to the sphincter muscle of the iris produces changes similar to those found in the case of tonic pupils. There is no proof that tonic pupils are dependent upon changes in the central nervous system or to disorder of the fibers of the sphincter muscle. No disturbance of sympathetic or parasympathetic innervation can be demonstrated. The tonic pupil is often irregular in outline suggesting a peripheral nerve injury.

The argument has been advanced that the position of the pupils in sleep represents an equilibrium of relation between dilator and constrictor forces. The point of relaxation of the sphincter muscle would lie at some point between maximal contraction and relaxation. The vascular tone of the blood vessels is relaxed during sleep. The small pupils of old age suggest a weakness of the dilator mechanism with normal power of pupillary constriction. If dilator and sphincter smooth muscles were involved, they would keep their opposing influence throughout life. Considering the blood vessels as the dilator mechanism, senile changes would explain the loss of dilator power.

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Plate II

Figs. 7 and 8. Photographs of the iris of both eyes of an albino rabbit. Figure 7 is from the normal eye. In the case of the iris shown in figure 8, the superior cervical ganglion had been removed 80 days earlier. The pupil is smaller in this iris with the sympathetic influence removed. There is also a greater injection of the blood vessels in the pupillary portion of the iris in figure 8 than in figure 7. This suggests that the smaller size of the pupil is dependent upon the greater filling of the vessels. In figure 7, portions of the choroid have been folded over the edge of the iris. Short ciliary arteries and veins enter the iris all around its lateral portion and have a part in making up the ciliary processes. The long ciliary vessels enter at the left upper and right lower portions of the photograph. The ciliary processes are of greatest complexity at the points farthest from the place of entrance of the long ciliary vessels into the iris × 6.

Plate III

Fig. 9. Photograph of the medial prolongation of a ciliary process in the iris. The large circular vessels run across the upper portion of the figure but are out of focus. In this case, the ciliary process appears to be contracted and coiled into a small space. × 84.

Fig. 10. The medial prolongation of the ciliary process in this case shows a loose arrangement of the blood vessels. The process extends downward toward the pupillary margin forming a pyramidal shaped structure. × 90.

Fig. 11. Medial prolongation of a ciliary process. The blood vessels making up the structure are tightly contracted to a circular mass in which the individual vessels cannot be made out. × 80.

Fig. 12. Blood vessels of the iris running medially from the ciliary region toward the pupillary margin. They are very much curved and have the appearance of corkscrews. × 80.

Plate IV

Fig. 13. The iris contains at least three plexiform layers of blood vessels which can be recognized by changing the focus of the microscope. One is shown here. Many of the nerves run along the blood vessels and assume the same pattern. They will be demonstrated as stained with methylene blue. × 86.

Fig. 14. The arrangement of the blood vessels at the pupillary margin of the iris. The pupil lies on the left. Many of these blood vessels are in the substance of the sphincter muscle. × 90.

Fig. 15. The ciliary processes of the iris are surrounded by an envelope of collagenous connective tissue fibers. This is demonstrated when the iris with the blood vessels injected with India ink is counterstained. × 90.

Fig. 16. The blood vessels of the iris itself injected with India ink showing the fibrous tissue supporting these vessels. The blood vessels form ridges in the pupillary portion of the iris which can be seen with the unaided eye. × 90.

Fig. 17. The venous drainage of the medial portion of the ciliary processes. The medial prolongation of a ciliary process is shown in the upper portion of the photograph. A vein arises in this area which extends downward and outward to empty into the circular vein of the iris at the lower left hand corner. × 90.

Fig. 18. The venous drainage of the pupillary portion of the iris. The vein extends outward to empty into the circular vein of the iris. × 90.

Plate V

Fig. 19. In this iris, the short ciliary vessels are injected with India ink but the long ciliary vessels did not stain. There was no ink in the circular artery and vein of the iris. The photograph indicates the form of the ciliary processes of the iris as injected through the short ciliary vessels. × 72.

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Fig. 11. Medial prolongation of a ciliary process. The blood vessels making up the structure are tightly contracted to a circular mass in which the individual vessels cannot be made out. × 80.

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Plate IV

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FIG. 20. The short ciliary arteries could be recognized inasmuch as the walls were thick and highly refractile. × 90.

FIG. 21. By changing the focus slightly, it was possible to demonstrate in the same material, the short ciliary veins of the iris which lie in a slightly different plane from the arteries. This figure shows the veins which accompany the arteries which are in focus in figure 20. × 90.

FIG. 22. An indication of the structure of the lateral prolongations of the ciliary processes in the preparation in which only the short ciliary vessels were injected. An artery gives rise to a fine network of arterioles and capillaries. On the opposite side of the process, the vein begins. × 90.

FIG. 23. An aneurysmal dilatation of a small vessel in the iris. There was no hemorrhage or escape of injection fluid into the surrounding tissue, and no indication that the blood vessels had been damaged by the pressure of the injection. × 90.

FIG. 24. Another local dilatation of a vessel in the iris giving the appearance of a small aneurysm. × 90.

FIG. 25. Elastic tissue fibers in the pupillary edge of the iris, stained with methylene blue. The pupillary margin lies at the upper edge of the picture. The dark fiber is an elastic tissue cell. The lower portion of the picture contains a characteristic nucleus of one of the elastic tissue fibers. × 360.

FIG. 26. In the central portion of the picture is the nucleus of an elastic tissue fiber. The cytoplasm of the cell can be seen less distinctly. × 360.

FIG. 27. A portion of the iris, stained with methylene blue showing nerve fibers and chromatophores in the mesodermal layer. × 280.

FIG. 28. This is a chromatophore with characteristic branching processes. These cells have often been mistaken for nerve cells. × 480.

PLATE VI

FIG. 29. Small arteries in the wall of the iris. The nuclei of the smooth muscle cells and sometimes the cytoplasm are stained with methylene blue. It will be noticed that the smooth muscle tends to be somewhat clumped and is thickest at the point where there is an angle in the wall of the artery. × 140.

FIG. 30. Smooth muscle in the wall of an artery of the iris stained with methylene blue. It shows the clumping of the smooth muscle cells at the point where the artery curves. × 160.

FIG. 31. A larger artery with the smooth muscle in its wall stained by the methylene blue technique. × 160.

FIG. 32. A small vein with staining of the nuclei of the endothelial cells. × 160.

FIG. 33. Capillaries stained with methylene blue. × 160.

FIG. 34. Large nerve trunks in the wall of the iris stained with methylene blue. The nerves in these trunks branch frequently and regroup themselves forming a plexus of nerves. × 200.

FIG. 35. Nerve trunks contained myelinated and unmyelinated fibers. × 310.

FIG. 36. Two medullated fibers, several smaller non-medullated fibers and chromatophores in the iris wall. × 280.

PLATE VII

FIG. 37. The myelinated fibers in the wall of the iris tend to branch many times. A number of small unmyelinated nerve fibers are shown. × 280.

FIG. 38. At points along their course, the myelinated fibers in the iris show localized enlargements of the axone. The significance of these swellings in the nerve is not known but they usually occur close to the nucleus of an endothelial cell in the wall of a blood vessel. The nucleus of the endothelial cell can be seen just above the local enlargement of the medullated fiber. There is fine, unmyelinated fiber at the upper edge of the picture. × 540.

Plate II

Figs. 7 and 8. Photographs of the iris of both eyes of an albino rabbit. Figure 7 is from the normal eye. In the case of the iris shown in figure 8, the superior cervical ganglion had been removed 80 days earlier. The pupil is smaller in this iris with the sympathetic influence removed. There is also a greater injection of the blood vessels in the pupillary portion of the iris in figure 8 than in figure 7. This suggests that the smaller size of the pupil is dependent upon the greater filling of the vessels. In figure 7, portions of the choroid have been folded over the edge of the iris. Short ciliary arteries and veins enter the iris all around its lateral portion and have a part in making up the ciliary processes. The long ciliary vessels enter at the left upper and right lower portions of the photograph. The ciliary processes are of greatest complexity at the points farthest from the place of entrance of the long ciliary vessels into the iris ×.6.

Plate III

Fig. 9. Photograph of the medial prolongation of a ciliary process in the iris. The large circular vessels run across the upper portion of the figure but are out of focus. In this case, the ciliary process appears to be contracted and coiled into a small space. × 84.

Fig. 10. The medial prolongation of the ciliary process in this case shows a loose arrangement of the blood vessels. The process extends downward toward the pupillary margin forming a pyramidal shaped structure. × 90.

Fig. 11. Medial prolongation of a ciliary process. The blood vessels making up the structure are tightly contracted to a circular mass in which the individual vessels cannot be made out. × 80.

Fig. 12. Blood vessels of the iris running medially from the ciliary region toward the pupillary margin. They are very much curved and have the appearance of corkscrews. × 80.

Plate IV

Fig. 13. The iris contains at least three plexiform layers of blood vessels which can be recognized by changing the focus of the microscope. One is shown here. Many of the nerves run along the blood vessels and assume the same pattern. They will be demonstrated as stained with methylene blue. × 86.

Fig. 14. The arrangement of the blood vessels at the pupillary margin of the iris. The pupil lies on the left. Many of these blood vessels are in the substance of the sphincter muscle. × 90.

Fig. 15. The ciliary processes of the iris are surrounded by an envelope of collagenous connective tissue fibers. This is demonstrated when the iris with the blood vessels injected with India ink is counterstained. × 90.

Fig. 16. The blood vessels of the iris itself injected with India ink showing the fibrous tissue supporting these vessels. The blood vessels form ridges in the pupillary portion of the iris which can be seen with the unaided eye. × 90.

Fig. 17. The venous drainage of the medial portion of the ciliary processes. The medial prolongation of a ciliary process is shown in the upper portion of the photograph. A vein arises in this area which extends downward and outward to empty into the circular vein of the iris at the lower left hand corner. × 90.

Fig. 18. The venous drainage of the pupillary portion of the iris. The vein extends outward to empty into the circular vein of the iris. × 90.

Plate V

Fig. 19. In this iris, the short ciliary vessels are injected with India ink but the long ciliary vessels did not stain. There was no ink in the circular artery and vein of the iris. The photograph indicates the form of the ciliary processes of the iris as injected through the short ciliary vessels. × 72.

FIG. 20. The short ciliary arteries could be recognized inasmuch as the walls were thick and highly refractile. × 90.

FIG. 21. By changing the focus slightly, it was possible to demonstrate in the same material, the short ciliary veins of the iris which lie in a slightly different plane from the arteries. This figure shows the veins which accompany the arteries which are in focus in figure 20. × 90.

FIG. 22. An indication of the structure of the lateral prolongations of the ciliary processes in the preparation in which only the short ciliary vessels were injected. An artery gives rise to a fine network of arterioles and capillaries. On the opposite side of the process, the vein begins. × 90.

FIG. 23. An aneurysmal dilatation of a small vessel in the iris. There was no hemorrhage or escape of injection fluid into the surrounding tissue, and no indication that the blood vessels had been damaged by the pressure of the injection. × 90.

FIG. 24. Another local dilatation of a vessel in the iris giving the appearance of a small aneurysm. × 90.

FIG. 25. Elastic tissue fibers in the pupillary edge of the iris, stained with methylene blue. The pupillary margin lies at the upper edge of the picture. The dark fiber is an elastic tissue cell. The lower portion of the picture contains a characteristic nucleus of one of the elastic tissue fibers. × 360.

FIG. 26. In the central portion of the picture is the nucleus of an elastic tissue fiber. The cytoplasm of the cell can be seen less distinctly. × 360.

FIG. 27. A portion of the iris, stained with methylene blue showing nerve fibers and chromatophores in the mesodermal layer. × 280.

FIG. 28. This is a chromatophore with characteristic branching processes. These cells have often been mistaken for nerve cells. × 480.

PLATE VI

FIG. 29. Small arteries in the wall of the iris. The nuclei of the smooth muscle cells and sometimes the cytoplasm are stained with methylene blue. It will be noticed that the smooth muscle tends to be somewhat clumped and is thickest at the point where there is an angle in the wall of the artery. × 140.

FIG. 30. Smooth muscle in the wall of an artery of the iris stained with methylene blue. It shows the clumping of the smooth muscle cells at the point where the artery curves. × 160.

FIG. 31. A larger artery with the smooth muscle in its wall stained by the methylene blue technique. × 160.

FIG. 32. A small vein with staining of the nuclei of the endothelial cells. × 160.

FIG. 33. Capillaries stained with methylene blue. × 160.

FIG. 34. Large nerve trunks in the wall of the iris stained with methylene blue. The nerves in these trunks branch frequently and regroup themselves forming a plexus of nerves. × 200.

FIG. 35. Nerve trunks contained myelinated and unmyelinated fibers. × 310.

FIG. 36. Two medullated fibers, several smaller non-medullated fibers and chromatophores in the iris wall. × 280.

PLATE VII

FIG. 37. The myelinated fibers in the wall of the iris tend to branch many times. A number of small unmyelinated nerve fibers are shown. × 280.

FIG. 38. At points along their course, the myelinated fibers in the iris show localised enlargements of the axone. The significance of these swellings in the nerve is not known but they usually occur close to the nucleus of an endothelial cell in the wall of a blood vessel. The nucleus of the endothelial cell can be seen just above the local enlargement of the medullated fiber. There is fine, unmyelinated fiber at the upper edge of the picture. × 540.

FIG. 39. A low-power photograph demonstrating the sphincter muscle at the pupillary margin and the parasympathetic fibers reaching the muscle. The pupillary margin shows at the lower edge of the photograph. Just above, a group of horizontal black dots indicate nerve endings upon the muscle fibers. Although the muscle itself is not stained, its position is indicated by the longitudinal striations. From above, groups of fine nerves extend down toward the sphincter muscle, finally dip beneath the surface, run longitudinally along the muscle fibers and give rise to the numerous dot-like endings. × 240.

FIG. 40. A photograph of higher magnification showing the entrance of the parasympathetic fibers into the sphincter muscle. The muscle fibers are indicated in the lower portion of the photograph by the horizontal striation. From above groups of fine nerve fibers extend downward; they then dip beneath the surface to reach the sphincter muscle. × 540.

FIG. 41. This indicates the great number of dot-like nerve endings which are found along the smooth muscle fibers of the iris sphincter. × 540.

FIGS. 42 AND 43. Myelinated fibers give rise to more complicated terminations, lying between the fibers of the sphincter muscle of the iris. In figure 43, the myelinated fiber may be seen in relationship to the complicated nerve ending. The bulbs making up the terminal plaques of these nerve endings are somewhat larger than the motor endings upon the muscle itself. × 540.

FIG. 44. A myelinated fiber terminating in a complicated ending in connection with the blood vessels near the pupillary margin. This is probably a sensory ending. It is extremely large and only a portion of it is shown in the photograph. Only small parts could be brought into focus at one time. In its entire extent, it covered almost the low-power field of the microscope. × 540.

FIG. 45. The stained nuclei of smooth muscle cells indicate the wall of a small artery in the iris. The artery is accompanied by nerve fibers which run across its surface and probably give rise to endings upon the artery wall. Similar endings, under higher magnification will be shown in other photographs. × 300.

FIG. 46. The chromatophores of the iris are in close contact with nerve fibers. This photograph shows a chromatophore with feet extending out close to a fine nerve fiber at the upper edge of the picture. × 320.

PLATE VIII

FIG. 47. The chromatophores orient themselves in relation to nerve fibers in the vicinity. The feet of the two chromatophores shown in the center of the photograph extend upward toward two myelinated fibers which cross the field near them. × 540.

FIG. 48. The foot of a chromatophore extending out to the vicinity of a nerve fiber close by. × 540.

FIG. 49. Dot-like endings of nerve fibers in the vicinity of chromatophores were found. It was difficult to bring the whole structure into focus. In the lower, left portion of the field, a nerve fiber, out of focus crosses toward the right and ends as a distinct black dot near the foot of a chromatophore. × 540.

FIG. 50. Nerve fibers running with the capillary plexus in the wall of the iris. The pattern of the small blood vessels has been shown in a previous picture. × 460.

FIG. 51. The plexus of nerves upon the walls of the blood vessels of the iris as seen with somewhat higher power. The plexus consists of small fibers and endings, extending along the vessel wall. × 600.

FIG. 52. In suitable places, it was possible to analyze the relationship of nerves to capillaries in the wall of a blood vessel. In the central portion of the figure is the nucleus of a cell making up the capillary wall and a dot-like enlargement of a nerve is seen ending near the nucleus. × 600.

FIG. 53. Small nerve fibers ending in plaque-like endings close to the nucleus of a cell in the capillary wall. × 600.

FIG. 54. A small blood vessel under high magnification. The ring shaped structures are

the nuclei of smooth muscle cells in the wall of the vessel In the lower central portion of the figure, a nerve ending, of plaque like type, may be seen just below the most complicated of the nuclei. × 620.

FIG 55 This is a low-power magnification giving an indication of the nerves upon the blood vessels of the ciliary body The lower darker portion of the photograph is the ciliary body and the small black dots indicate nerve fibers running along and ending upon the blood vessels × 210

FIG 56 Low power magnification showing nerve fibers in the mesodermal stroma of the iris wall × 210

PLATE IX

FIG 57 An artery is running horizontally across the photograph as evidenced by the ring like nuclei and the smooth muscle fibers At the upper edge of the artery a nerve ending can be seen in close relationship with a smooth muscle cell × 400

FIG 58 The nerves and nerve endings in the wall of a small artery, the circular artery of the iris At the lower edge of the picture, slightly out of focus can be seen a trunk of nerve fibers extending upward to enter the wall of the artery The fibers terminate in characteristic endings upon the smooth muscle and in connection with the endothelial cells × 260

FIG 59 Endings upon the endothelial layer of a small artery The endings are arranged in a more diffuse pattern than those upon the smooth muscle × 500

FIG 60 These are nerves giving rise to endings upon the smooth muscle of a medium sized artery, the circular artery of the iris × 500

FIG 61 The pattern of nerve fibers in the choroid layer of the eye The nerve fibers follow the blood vessels of the choroid giving rise to the dot like endings upon the blood vessels, especially in connection with the nuclei of their wall × 500

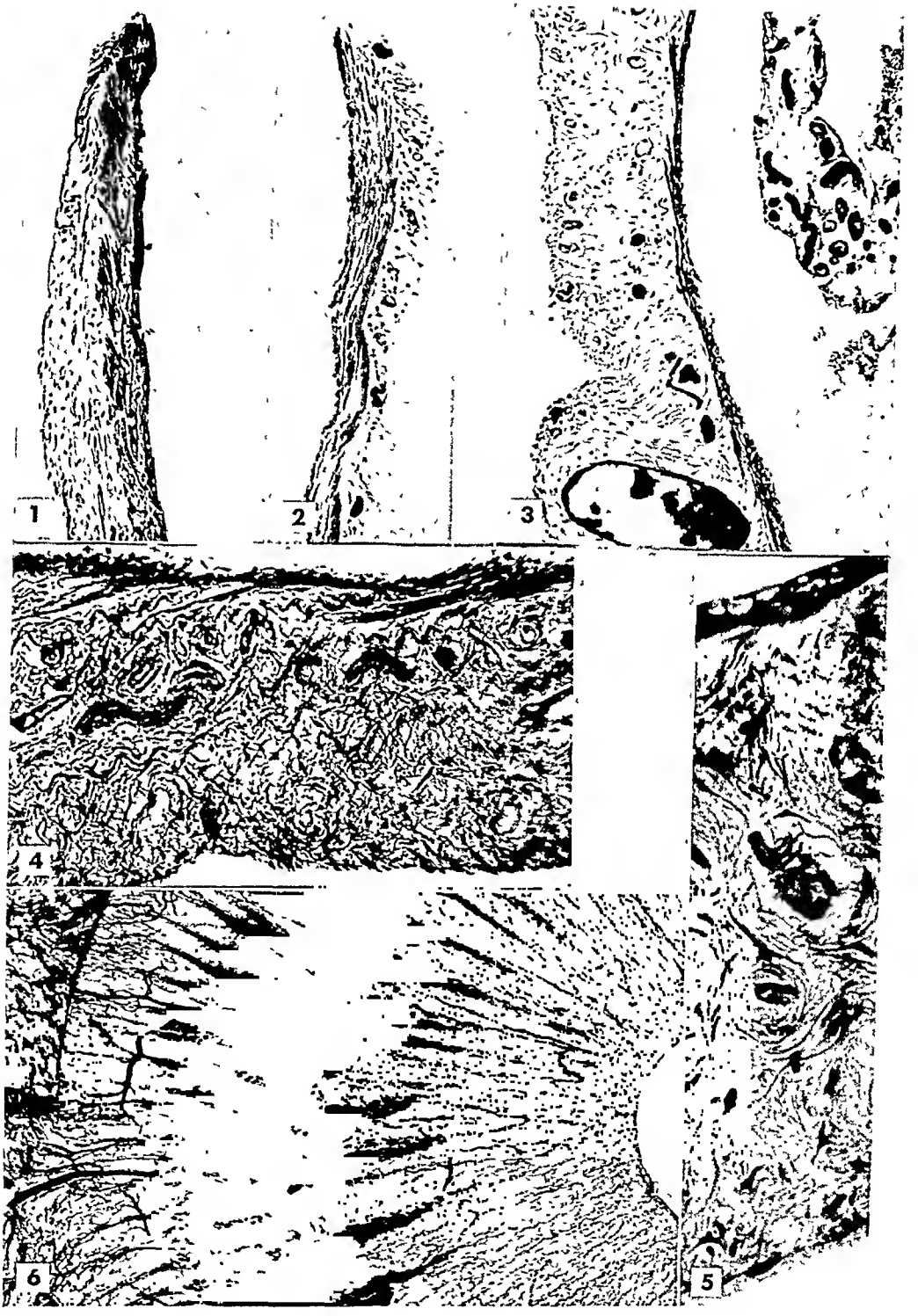
FIG 62 In the center of this picture, the dark area indicates the nucleus of an endothelial cell in the capillary wall of the choroid Enlargements of the nerve fibers producing bulbous nerve endings can be seen adjacent to the nucleus × 500

FIG 63 A myelinated fiber gives rise to complicated branching nerve endings upon the wall of small blood vessels near the pupillary margin This is probably a sensory ending × 500

FIG 64 This is a higher magnification, showing the endings of a myelinated fiber Some of the endings are in close connection with the nucleus of a cell in the wall of a blood vessel × 500

FIG 65 The complicated terminations of a myelinated nerve fiber upon the wall of a small blood vessel near the pupillary margin These endings are probably sensory × 500

FIG 66 This is a local dilatation found along the course of a myelinated fiber in the iris which is in close connection with an endothelial cell in the wall of a blood vessel × 500.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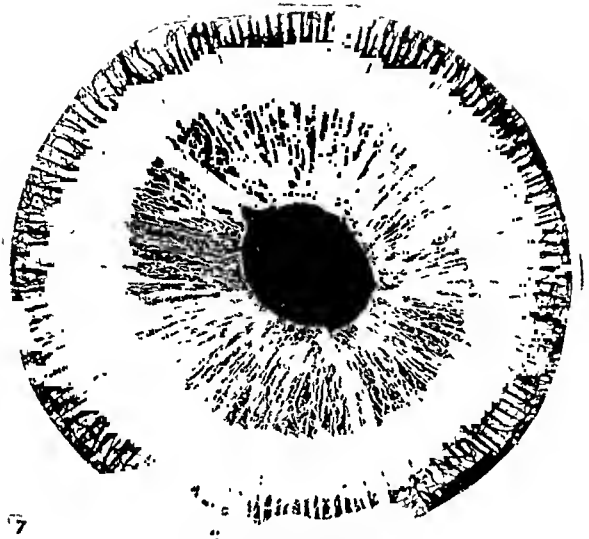

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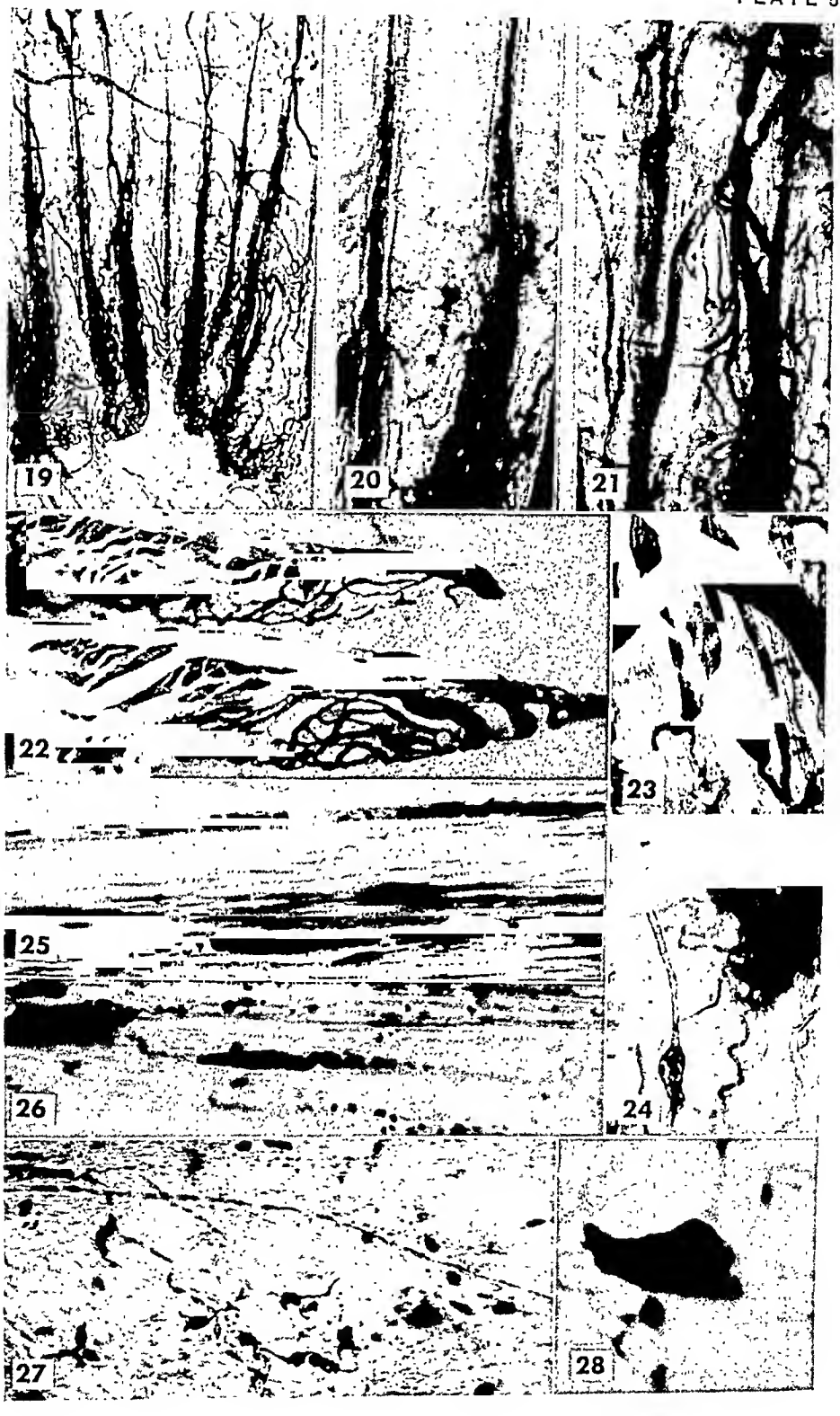
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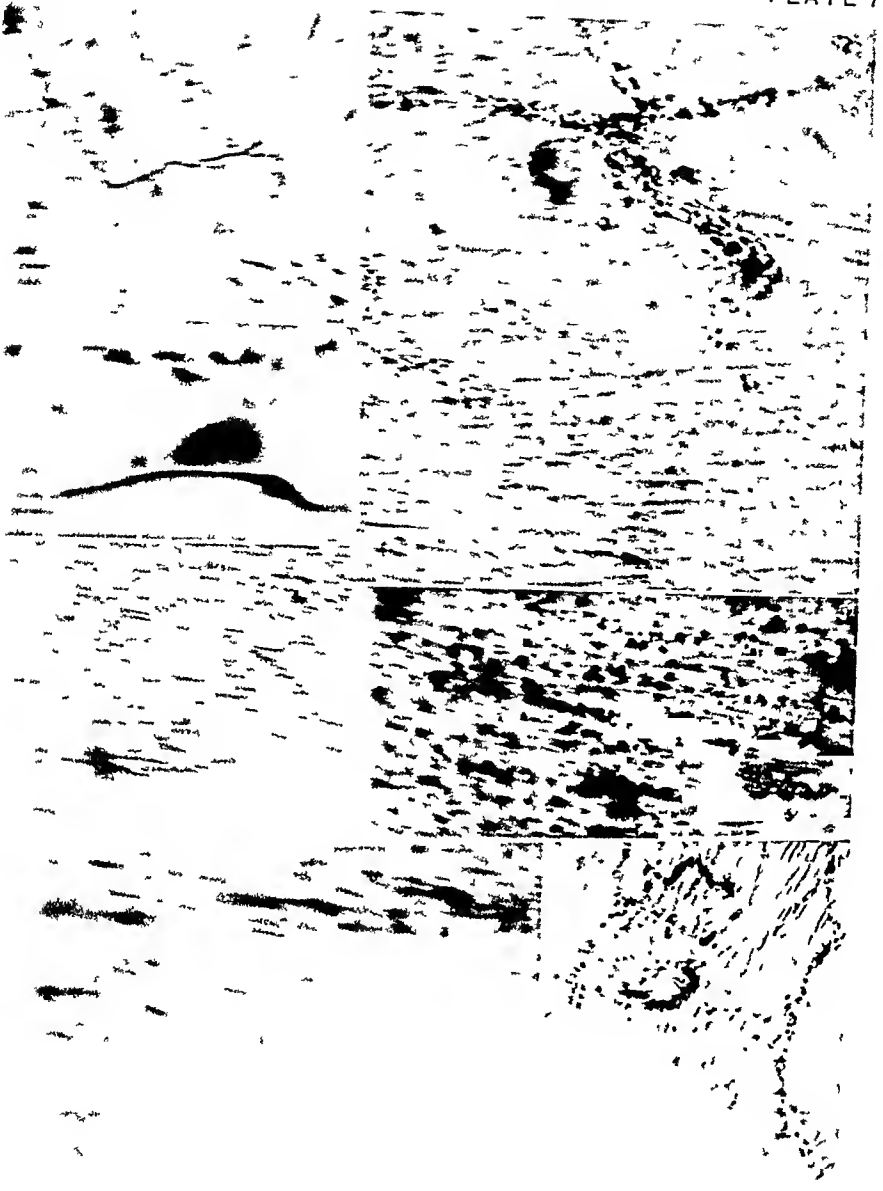
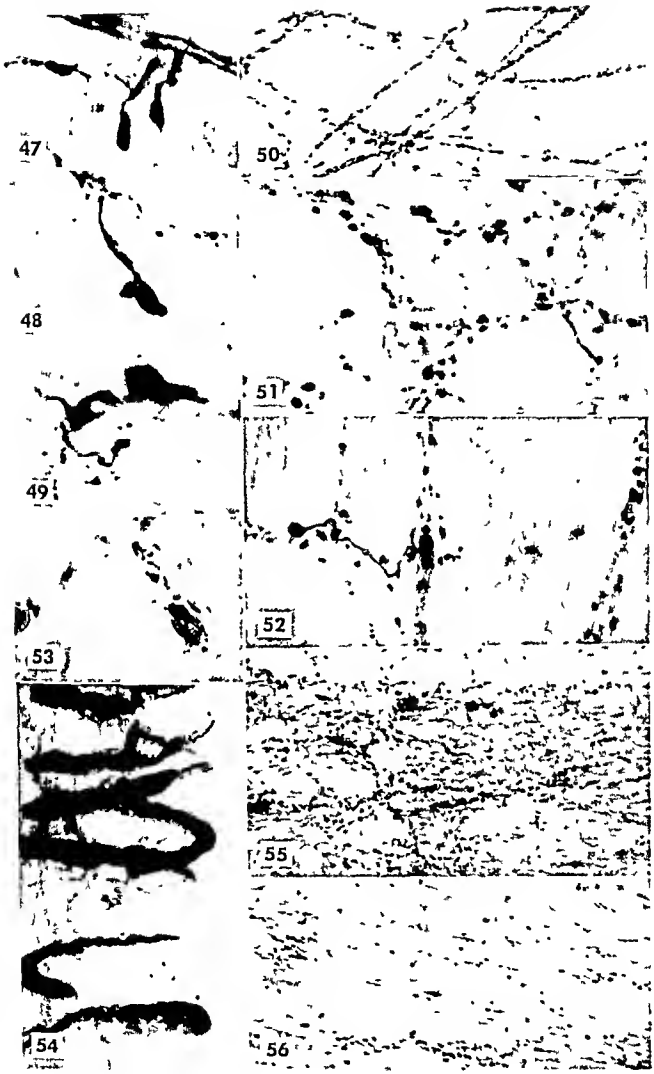
PLATE 7

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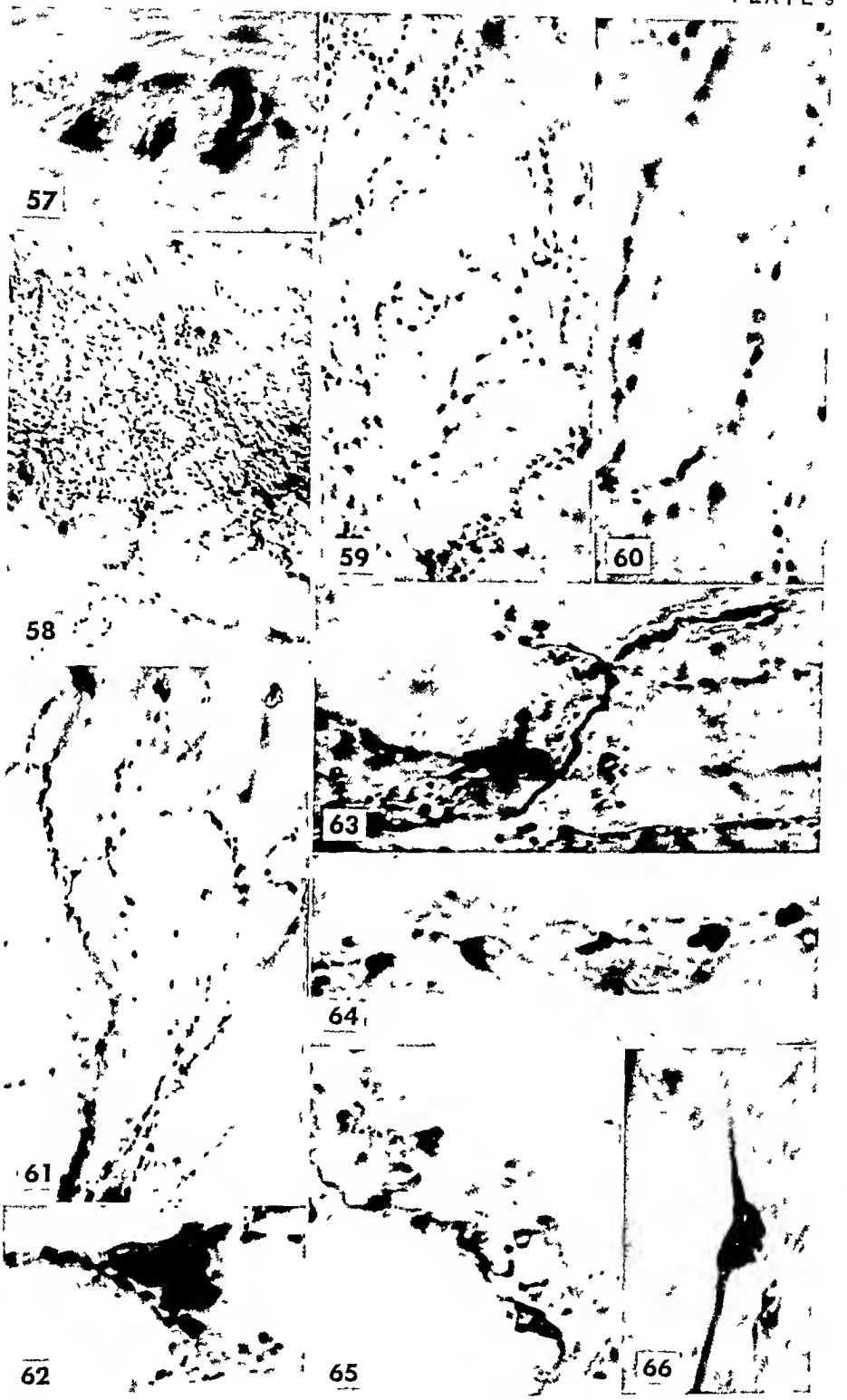
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COLD HEMAGGLUTINATION—AN INTERPRETIVE REVIEW[1]

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Introduction

The literature of agglutination of red blood cells in the cold is confusing because of inaccuracies in definition and terminology. In many instances the interpretation of experimental and clinical data is not clear and direct. The serological characteristics of the cold hemagglutination reaction may be categorically stated as follows:

1. A mixture of red blood cells and serum exhibits agglutination in the cold (best demonstrated between 5 and 0°C.). At 37°C. and usually at temperatures over 25°C. agglutination is not observed.
2. The phenomenon of agglutination in the cold can be reversed by warming to temperatures over 20 to 30°C. and again reversed to the original agglutinated state by cooling to below 10 to 20°C.
3. The antibody in such a serum agglutinates all human red blood cells regardless of group; slight irregular variations in the intensity of the agglutination may be observed.
4. The agglutinating serum is also active in varying degree against the erythrocytes of many unrelated species.
5. A serum with cold hemagglutinins can be exhausted by appropriate absorptions with erythrocytes in the cold.
6. Absorbed agglutinins can be released from agglutinated erythrocytes by raising the temperature to 37°C.
7. Like other agglutinins, the cold hemagglutinin resists storage in the cold with only slight diminution in potency.
8. Cold hemagglutinins do not lose their activity after heating to 56°C. for 30 minutes.
9. Other unusual atypical cold agglutinins with varying specificity are described under the paragraph heading "Irregular Cold Hemagglutination."

Terminology

The terms autoagglutination, panagglutination, and pseudoagglutination have been used at one time or another to describe this reaction. None of these is suitable.

AUTOAGGLUTINATION

Agglutination reactions with the above characteristics are commonly called autoagglutination. This term was popularized by the French and Italian writers as "La Grande Autoagglutination." It is immediately apparent that such a term describes only one aspect of this reaction, namely, agglutination of erythrocytes by their own serum. It leaves unmentioned the effect of temperature, the reversibility, and the relative lack of specificity which the agglutinin possesses. Furthermore, the term autoagglutination, when applied to this reaction, presupposes that any serum which agglutinates homologous erythrocytes has the characteristics described above. While this is true in most instances, sera have been described which agglutinate homologous cells but do not possess the other properties (see paragraph "Irregular Cold Hemagglutination"). As has been vigorously pointed out by Mino (121), the term autoagglutination implies a specificity that in no way is borne out by the facts. The striking feature of the

reaction is not that the agglutinin acts on its own cells but that it acts on so many related and unrelated red blood cells with little difference in intensity.

PANAGGLUTINATION

Panagglutination was suggested by Mino (121) as a convenient appellation for a phenomenon in which lack of specificity is a striking characteristic. There are no other antibodies whose scope of action may be so broad; there are few other instances in which species boundaries are so completely disregarded as in the case of the cold agglutinin. The reaction of cold hemagglutination is like agglutinating and hemolysing substances of plant origin, abrin and ricin, or snake venoms, whose actions are almost universal. From the immunologic point of view the term panagglutination is a desirable one, even though some differences in susceptibility to agglutination exist. All human erythrocytes contain the receptors for human cold hemagglutinins; among animal erythrocytes considerable variation in receptors for human cold hemagglutinins apparently exists. Different human sera have acted differently on animal erythrocytes (1, 26, 27, 169 and others). More information on this point is desirable.

As compared to other antibodies whose activity is restricted to the antigen used in immunization (or to closely related antigens), the lack of specificity of the antibody described herein entitles it to the term of panagglutinin. The entire question of the specificity of antibodies is thoroughly discussed by Landsteiner (90). He points out that, even among hemagglutinins of plant origin whose activity on many unrelated species erythrocytes is broad, a certain type of specificity exists. There apparently are no antibodies which act against all antigen complexes equally.

Panagglutination has also been used to describe an entirely different phenomenon—the Hübener-Thomsen phenomenon. This is unrelated to cold hemagglutination and is manifested in red blood cells that have been acted upon by certain bacteria whereby they obtain the property of being agglutinated by almost any serum (48). An agglutinin present in all normal sera acts upon an agglutinogen which lies dormant in the erythrocytes; bacterial action upon the cells reveals the antigen. All descriptions (161) stress the fact that old (over 12 hours) erythrocytes must be used. Though this peculiar agglutination differs from cold hemagglutination, they in common possess somewhat similar reactions to temperature. Cold hemagglutination, Hübener-Thomsen agglutination, and isoagglutination all have higher titers at low temperatures. The titers fall off rapidly with increasing temperature so that cold hemagglutination rarely occurs above 25°C.; the Hübener-Thomsen agglutination may occur at 37°C. (its end point is variable); isoagglutination[5] occurs up to 45–50°C. Lattes and Crema (97) stated that the agglutinin of the Hübener-Thomsen phenomenon might be identical with the cold hemagglutinin. Friedenreich (48) amply contraverted this when he showed that the cold hemagglutinin could be completely absorbed from a serum without affecting the other antibody.

[5] Isoagglutination is the agglutination of the erythrocytes of an individual by the blood serum of another individual of the same species. In humans isoagglutinins and isoagglutinogens are concerned in blood grouping.

PSEUDOAGGLUTINATION OR ROULEAUX FORMATION

Except for unusual cases, most of the early and even some of the more (130) recent literature on the subject of cold hemagglutination is confused by the inclusion of the phenomenon of rouleaux with that of agglutination. Recent texts on hematology use the term autoagglutination and imply cold hemagglutination in discussing the rouleaux formation in multiple myeloma (70, 189, 198).

Actually there are no similarities between agglutination and rouleaux formation. The use of the term autoagglutination to describe the clumping tendency of the erythrocytes in multiple myeloma and other conditions characterized by an elevation in the serum globulin is entirely unwarranted and should be discontinued. In such states excessive rouleaux formation is exceedingly common and is of diagnostic importance. The reader is referred to Wiener's text (194) and Levine (103) for a clear exposition of the differences between cold hemagglutination and pseudoagglutination or rouleaux formation. Because of this confusion in terminology, it is often difficult to interpret correctly some of the reported cases. Several writers, especially Lattes (96) and Neuda (137), have described rouleaux formation as the first stage of cold hemagglutination and believe that some relationship exists between these processes. This was not observed in the cases studied by us and is apparently not upheld by the majority of authors (122). Thomsen (177) stated that there is some relationship between cold hemagglutinins and rouleaux formation since high titers of the former tended to occur in sera with marked rouleaux formation.

As pointed out by Fahraeus (45) and many other authors, pseudoagglutination is non-specific, dependent in large measure upon globulin and fibrinogen concentrations in blood. With increases of these plasma proteins, rouleaux formation becomes more marked. It is decreased when spherocytosis of the erythrocytes is present. Pseudoagglutination disappears on slight dilution of the plasma and is inhibited by the presence of lecithin. It occurs best at 37°C. but also occurs in the cold. Storage for a short time destroys the phenomenon. A plasma with pseudoagglutinating properties cannot be exhausted by repeated absorptions with erythrocytes.

COLD HEMAGGLUTINATION

The term cold hemagglutination focuses attention upon the relationship of the reaction to temperature. This is a fundamental property of the agglutinin; it is of importance clinically and experimentally. There are no other antibodies whose activities are so dependent upon temperature changes. It suffers only from criticisms that other agglutinins act more strongly in the cold than at 37°C. (see above), and that in some of the observed instances of the phenomenon under discussion, agglutination of the cells, though most marked below 5°C., was also observed at 25°C. or higher.

In the following discussions the term "cold hemagglutination" will be used as the one most completely descriptive of the phenomenon in its entirety. The expression "autoagglutination" will be reserved to describe only one property

of cold hemagglutination, i.e., agglutination of erythrocytes by homologous serum.

IRREGULAR COLD HEMAGGLUTINATION

Landsteiner and Levine (89) have reviewed previously published studies on irregular or atypical human hemagglutination reactions and have presented exhaustive data on these rare reactions. They described an intermediate type of agglutinin whose action is distinct at 20°C. and only exceptionally persists at 37°C. Some of these irregular cold agglutinins have group specificity differentiating A2 from A1 cells. Kettel (82) has summarized the subject and has described three types of cold hemagglutinins. The first is the common variety which we are considering in this publication which acts on all human and many animal erythrocytes. The other types are extremely rare. One, cold agglutinin $\alpha 1$, acts strongly on A1 cells and weakly on O and A2 cells; the other, cold agglutinin $\alpha 2$, acts strongly on O and A2 cells but weakly on A1 cells. Landsteiner and Levine (91) stated that cold agglutination is not an entirely satisfactory method for detecting subtle differences in individual human blood. Other irregular or atypical cold agglutinins are described in all the blood groups by Landsteiner and Levine. A classification of these reactions is not possible at present (see 39).

Ham and Castle (63) in a case of acute hemolytic anemia described extreme autoagglutination in vitro at 37°C. and a high concentration (1:1025 at 0°C.) of cold hemagglutinins in the serum. Wiener (193) described a fatal case of acute hemolytic anemia in which an autoagglutinin titer of 1/64 was observed at 5, 20, and 37°C. In the case of Reisner and Kalkstein (147) agglutination was present at 37°C. in a fatal case of hemolytic anemia. An even suspension of cells could be obtained only by washing with saline solution at 50°C. In the case of Hochheim and Rosenthal (72) variable agglutination was observed at 37°C. D'Antona (33) stated that dispersion of agglutinated cells did not occur unless the temperature was raised at 45–50°C.

In Mino's (120) second case of hepatosplenomegaly with a high titer of cold hemagglutinins, agglutination was observed at 37°C., though much reduced in strength. Aubertin (2) stated that autoagglutination persisted even at 37°C. He described two kinds of autoagglutination. The first is weak and occurs in acquired hemolytic anemia and trypanosomiasis. The second is powerful and rare and is called "La Grande Autoagglutination." In three of the cases of Vogel, Rosenthal and Levine (182) cold agglutinins were found. In cases 1 and 4, anti Rh agglutinins in an Rh negative patient were also present. These were responsible for the transfusion reactions. In case 7 hemolytic transfusion reactions were not due to Rh incompatibility. In this case an atypical agglutinin was present. This acted on 60 per cent of all group O erythrocytes, predominantly in the cold. The titer was high at 4°C. but distinct agglutination was observed at 37°C. When fresh serum (containing complement) was used at 37°C., hemolysis was observed; with inactivated serum only agglutination

occurred. Other case reports of irregular cold hemagglutinins are cited b Neter (136) and Ottenberg and Johnson (143). This summary is only a parti review of the literature on these perplexing problems. The agglutinin ar hemolysin observed by Dudgeon and Wilson (41) in a case of hepatic cirrhos due to syphilis may fall into this group.

Some of the early cases which are now included in the group of cold hemaggl tination were encountered in the course of other investigations. In thes agglutination was mentioned but not discussed. Experiments were not co ducted to elucidate the characteristics of the agglutination phenomenon. F this reason it is not possible to be certain of their nature. When there was co siderable doubt about the case or the phenomenon described, we eliminated from consideration. We have collected these references, however, and t reader is referred to the individual papers for more detailed information. Son of these cases have been included in the group of cold hemagglutination l previous writers.

The following publications were reviewed but are excluded from the tat because cold hemagglutination did not seem likely. They are included here make the compilation complete.

Lattes (95) studied the serum of a patient with angioneurotic edema a polyuria on exposure to cold. Though he used the term 'autoagglutination', l *illustrations depict rouleaux formation.*

In Rist's (150) first case he described autoagglutination unaffected by temper ture in a case of senile pruritus. The patient's serum did not agglutinate norm red blood cells. In his second case he mentioned that a red blood count cou not be performed in a young woman with chlorosis.

Engel (44) described erythrocytes as abnormally sticky in 5 cases of anemia undetermined origin.

Friedmann (50) described 3 cases of agranulocytosis which recovered wi roentgen therapy. In one a red blood count could not be done in Hayem solution because of agglutination of the red blood cells.

Roth (156) described a typical severe case of pernicious anemia in whi autoagglutination and autohemolysis were seen occasionally.

Rubaschewa (159) described a healthy individual with autoagglutinatio No details were given.

Bond (17) presented a confusing description of autoagglutination in pneumoni

Clinical Aspects

In the following summary (Table I) of all of the reported cases of cold her agglutination in disease, the clinical and experimental data of each presentatic were analyzed critically. When these conformed in their essentials to the prese views on agglutination, they were included. In those marked "data insufficient it was not possible to be certain that the phenomenon of cold hemagglutinatic was under discussion. Included by other writers within this group are exampl of cold hemagglutination in normal individuals. These have been excluded.

THE FIRST DESCRIPTION OF THE REACTION[6]

Cold hemagglutination was first described by Landsteiner (92) in 1903 in reactions between the red blood cells and sera of normal guinea pigs, chickens, horses, dogs, rabbits, and cattle. In that communication he set forth the most important characteristics of the phenomenon, namely the striking effect of low temperature, the disappearance of agglutination with a rise in temperature, the absorbability of the agglutinin in the cold and its release in the warm. He prepared saline solutions of almost pure agglutinin, after removal of the supernatant serum, by exposing cells agglutinated in the cold to moderate heat in the presence of normal saline solution. In 1899 Kanthack, Durham and Blandford (79) in a report on trypanosomiasis stated: "Instead of forming rouleaux, the red corpuscles tend to clump into masses and to lose their outlines, especially when the anemia is pronounced (rabbit, ass, and horse)."

The first definite description of the same phenomenon in humans was made by Biffi in 1903 (13) who observed the occurrence of hemagglutination in pneumonia, malaria, and the early stages of Oroya fever. He stated, "I have repeatedly observed a fairly high agglutinating power of some pathological serums for their own red cells." Careful reading of his paper leaves no doubt that he appreciated the differences between rouleaux formation and true agglutination. This cannot be said for other authors of his time.

Many French writers referred to Hayem (67) as the one to have made the pioneer observation. This reference concerns a single sentence in his text of 1899 in which he mentioned the occurrence of erythrocytes in "piles of money" in a case of cirrhosis of the liver. Reitmann (1890) (148) has been given credit by some. In a case of biliary cirrhosis of the liver "one of several blood counts could not be performed because the red blood cells were sticky and pasted together in three's and four's." Many continental authors have given Klein (83) the credit for describing the phenomenon. In 1902 he pointed out that a case that he had described in 1890 (84) showed hemagglutination, though the

[6] We are greatly indebted to Dr. Camille Dreyfus for the following citation in which hemoglobinuria following exposure to cold is described. This may be the first reference to this phenomenon. There are no details which permit more exact identification of the case.

From: Operum Io. Baptistae Montani (1498–1551) Veronensis Philosophi ac medici longe praestantissimi Editio Basileae. Tom. II, Liber I, Cap. VII De nigritudine urinae.

nisi præcedentibus aliquibus morbis fortissimis. Sã
guis etiam niger efficitur ab extrinseca causa, vt in
cancro. nam propter aërem frigidum, vel propter
percussionẽ: vel potest esse, quòd torrefiat sanguis,
qui deinde assatus deuenit ad nigredinem, vt mani-
festè apparet in carbunculis, quando per cutim dis-
pergũtur. Quando igitur apparebit vrina nigra me
rito sanguinis nigrefacti, cognoscetur quando pre-
cesserit frigiditas, & refrixerit sangũis intra venas,
qui maximè refrigeratus, postea à valida virtute trãs
mittitur vel per secessum, uel per vrinam. Cognoscẽ

term was not used at that time. The article in which that case was described concerned the counting of erythrocytes. He stated that a count could not be performed in a case of hypertrophic cirrhosis of the liver because of an abnormal cohesion of the blood cells. The cells were agglomerated and the boundaries of the individual cells could not be distinguished. He made no mention of any other properties which might identify the reaction. Italian writers have often mentioned Ascoli (6). Though he used the term agglutination, he presented no data by which the reaction could be identified. In 1929 Aubertin, Foulon and Bretey (7) referred to a case of cirrhosis of the liver observed in 1904 in which autoagglutination prevented enumeration of the red blood cells. This case had not been described in any publication by the authors. A careful critical analysis of the historical beginnings of cold hemagglutination was presented by Mino (123, 125) who concurred with the views entertained here.

Subsequent writers on this subject did not clarify any of the points established by Landsteiner until 1918 when Clough and Richter (26) carried out an exhaustive series of studies on a case of cold hemagglutination associated with pneumonia. Very little has been added to the subject since that publication.

Clinical Laboratory Abnormalities Which May Indicate the Presence of a Cold Hemagglutinin

It should be noted that the diagnoses in these tabulated cases are quite varied, ranging from acute infectious processes to Raynaud's syndrome. In many (exclusive of hemolytic anemia), anemia of some variety was present.

Red Blood Count. Often the cold hemagglutination is first observed during the performance of a red blood count. When the agglutinin titer is high, clumps of cells are visible grossly in the pipette after dilution preliminary to counting. With a lower titer the clumping may be observed only microscopically after the contents of the pipette have been discharged upon the counting chamber. Clumping is also visible on the stained smear.

If agglutination or clumping is observed during the performance of a red blood count using sodium citrate or normal saline solution as the diluent, slight warming of the reagents and apparatus will either prevent clumping or will disrupt clumps which are present. With other diluting fluids containing heavy metals (Hayem), globulin precipitation, not reversible by warming and not accentuated by cooling, may be observed in cases of hyperglobulinemia. This is not to be confused with cold agglutination.

Cold hemagglutinins do not interfere with hemoglobin determinations by the acid hematin method. Complete hemolysis of the blood is observed after it has been added to and mixed with the acid. Rarely, when clumping or precipitation is noted during this test, it is probably due to protein (globulin) precipitation in cases of hyperglobulinemia.

Blood Grouping. Frequently cold hemagglutination is first suspected when unexpected findings result from blood grouping determinations. The patient's red cells absorb cold hemagglutinins from their serum, especially if the blood is stored in the refrigerator. Such red cells will often remain agglutinated at room temperature. If they are mixed at room temperature with anti-A and anti-B

TABLE I

Reported Cases of Cold Hemagglutination

AUTHOR	YEAR	TITER	DIAGNOSIS AND REMARKS
Klein (84)	1890		Cirrhosis of the liver; data insufficient.
Ascoli (6)	1901		Data insufficient; no details.
Eisenberg (43)	1903		B. Pyocyaneus sepsis; anemia; data insufficient.
Biffi (13)	1903		Malaria, pneumonia, and in early stages of Oroya fever (Bartonellosis).
Christy (24)	1904		Trypanosomiasis (no case reports).
Martin and LeBoeuf (111)	1908		Trypanosomiasis (no case reports).
Widal, Abrami and Brulé (191)	1908		Same as Brulé (1909). Agglutinin in one case disappeared with spontaneous cure of anemia.
Brulé (19)	1909		5 cases of acquired hemolytic anemia; 1 case of icterus of hepatic origin (summary of the work of early French writers).
Todd (178)	1910		Trypanosomiasis (no case reports).
Yorke (201)	1910		Trypanosomiasis (no case reports).
Dubois (40)	1912		Trypanosomiasis (no case reports).
Antonelli (4)	1913		Acquired hemolytic anemia; data insufficient; cured by splenectomy; fragility of red cells increased..
Clough and Richter (26)	1918	1/500	Pneumonia and rheumatic heart disease; review of the subject; titer fell to 1/10 during convalescence.
Lüdke (107)	1918		3 cases of hemolytic anemia; data insufficient.
Kligler (85)	1922	1/160	Pregnancy and bleeding hemorrhoids; newborn did not show cold hemagglutinins.
Cohen and Jones (27)	1924	1/162	Pernicious anemia; hemoglobin 44 per cent; erythrocyte saline fragility normal.
Debenedetti, V. (36)	1924	1/60	Hemolytic anemia; fragility of erythrocytes decreased.
Mino (121, 123, 125)	1924	1/1000 to 1/3000 1/2000 to 1/10,000	2 cases of hepatosplenomegaly; positive Wassermann reactions; data insufficient.
Claveaux and Sanchez (25)	1924	1/10	Pneumonia; data insufficient.
Michon (117)	1925		Chronic bronchitis; data insufficient.
Greppi (59)	1925		Infectious cholangitis (cases 1 and 3).
Altounyan (8)	1925		Marked splenomegaly in chronic estivo-autumnal malaria; disappearance of cold agglutination after splenectomy.
Iwai and Nin (76)	1925	1/1000	Raynaud's syndrome.

TABLE I—*Continued*

AUTHOR	YEAR	TITER	DIAGNOSIS AND REMARKS
Alexander and Thompson (1)	1925	1/1280	Lymphatic leukemia; hemoglobinuria and sensitivity to cold; hemoglobin 20 per cent.
Amzel and Hirszfeld (3)	1925	1/16	Hemoglobinuria (famous case of Serbian soldier).
Li Chen-Pien (104)	1926	1/5	Cirrhosis of liver; titer low; cold hemagglutinin present in ascitic fluid; syphilis.
Wyschegorodzewa (200)	1926	1/1600	Hemolytic anemia; autohemolysin present; hemoglobin 65 per cent; erythrocyte saline fragility increased.
Stillman (173)	1926	1/640	Pneumonia and bacteremia; history of malaria.
Iwai and Nin (77)	1926	1/2000	Raynaud's syndrome.
Milders (119)	1928		Hypertrophic cirrhosis of the liver, 3 cases; ascitic fluid contained cold hemagglutinin.
Stieffel (172)	1928		Case 4—hemoglobinuria and sensitivity to cold; case 3—old malaria; isohemolysis present; data insufficient.
Aubertin, Foulon and Brotoy (7)	1929	1/300	Hemolytic anemia with thrombopenia and normal fragility of the erythrocytes. Case of cirrhosis of the liver.
Montanari (128)	1929	1/90 1/100 1/900	Case 1—diabetes; case 40—glomerulonephritis; case 29—alcoholic cirrhosis of the liver, fragility of red blood cells normal, Van den Bergh reaction direct positive, carcinoma of larynx.
Della Volta and Azzi (38)	1929	1/1100	Case 3—no definite diagnosis.
Greppi (60)	1929		Cholangitis lenta with splenomegaly.
Debenedetti, E. (35)	1929	1/80	Data insufficient. Case 1—urticaria and asthma, eosinophilia; case 2—hemolytic anemia, hemoglobin 33 per cent, erythrocyte saline fragility increased; case 3—sister of 2, erythrocyte saline fragility increased.
D'Antona (33)	1930	1/1280	4 cases of cholangitis lenta with splenomegaly (unusual thermal amplitude).
Lloyd and Chandra (105)	1930		Anemia; data insufficient.
Hochheim and Rosenthal (72)	1930		Probable hemolytic anemia, thrombocytopenia, variable agglutination present at 37°C.
Stewart and Harvey (171)	1931		Case 1—post hemorrhagic (?) anemia, hemoglobin 18 per cent; case 2—thrombopenic purpura and anemia cured by splenectomy.
Van der Hoeden and Verbeek (181)	1931	1/10 to 1/10	4 cases of hypertrophic cirrhosis of the liver.
Boxwell and Bigger (18)	1931	1/20	Atypical leukemia.

TABLE I—*Continued*

AUTHOR	YEAR	TITER	DIAGNOSIS AND REMARKS
Davidson (34)	1932		Macrocytic hemolytic anemia; increased fragility of the erythrocytes.
Misawa and Ohta (126)	1932		Syphilis, bronchial asthma, and chronic nephritis (data insufficient).
Gonzalez-Guzman (55)	1932		No definite diagnosis; hemoglobin 80 per cent; amebiasis and malaria.
Troisier and Catton (180)	1932		Hemolytic anemia; data insufficient.
Brulé, Hillemand, and Bonnard (20)	1933	less than 1/100	Cirrhosis of the liver; reversal of A:G ratio; positive formol gel test.
Manheims and Brunner (110)	1933		Myelogenous leukemia and severe anemia.
Benhamou and Nouchy (10)	1933		After splenectomy for malaria, cold hemagglutination developed. When the cold agglutination was dissipated by heating to 40°C., chilling did not reproduce hemagglutination; data insufficient.
Riebeling (149)	1933	1/200	Pernicious anemia.
Lewin (99)	1934		Cirrhosis of the liver (Hanot type); erythrocyte saline fragility normal.
Sherman (167)	1934	1/16	Case 1—pneumonia.
		1/32	Case 2—staphylococcal sepsis.
		1/32	Case 3—pneumonia.
Thiodet and Ribère (175)	1934		Data insufficient; diagnosis not given.
Roth (155)	1935		Hemoglobinuria and sensitivity to cold; oxyhemoglobin shown spectroscopically in serum.
Salém (160)	1935	over 1/512	Probable hemolytic anemia; hemoglobinuria and sensitivity to cold; normal fragility of erythrocytes to hypotonic saline.
Belk (9a)	1935	1/64	Lobar pneumonia.
Koepplin (86, 87)	1935	over 1/100	Case 1—pernicious anemia; hemoglobin 40 per cent.
		1/512	Case 2—hemolytic anemia and hemobloginuria; erythrocyte saline fragility increased; familial hemolytic anemia.
Patterson and Smith (145)	1936		Acute hemolytic anemia; cold hemagglutinin present for only 5 days; fragility of red cells normal.
McCombs and McElroy (115)	1937	1/1024	Hemoglobinuria; sensitivity to cold and gangrene; RBC 3,040,000; erythrocyte saline fragility normal.
Giordano and Blum (54)	1937		Case 2—acute hemolytic anemia; cold hemagglutinin persisted 3 weeks.
Rosenthal and Corten (153)	1937	1/40	Hemolytic anemia; fragility of erythrocytes increased; purpura.

TABLE I—*Continued*

AUTHOR	YEAR	TITER	DIAGNOSIS AND REMARKS
Greenwald (58)	1938		Case 2—acute hemolytic anemia; date insufficient; cold hemagglutinin present for 5 days.
Michon, Grandpierre, and Verain (118)	1938		Chronic bronchitis; pulmonary emboli or thromboses.
Hanns and Sommer (65)	1938		Acrocyanosis; hemoglobinuria; previous urticaria.
Sézary, Kipfer, and Gharib (166)	1938		Hemolytic anemia; Raynaud's syndrome; data insufficient.
Watson (184)	1939		Acquired hemolytic anemia; erythrocyte saline fragility normal; mentions another case.
Antopol, Applebaum, and Goldman (5)	1939		Case 1—pneumonia; sulfanilamide; hemolytic anemia. Case 2—tonsillitis; sulfanilamide; hemolytic anemia. Cold hemagglutinins disappeared during recovery.
Galli and Mussafia (51)	1939	1/600	Chronic cirrhosis of the liver; alcoholic.
Sedallion and Monnet (165)	1939		Kala azar; data insufficient. Cold hemagglutination transient.
Scott and Meerapfel (163)	1939		Sulfonamide hemolytic (?) anemia in staphylococcal sepsis and scarlet fever; cold hemagglutination transient.
Wheeler, Gallagher, and Stuart (187)	1939	1/16,000	Pneumonia; blood transfusions; agglutinin transient.
Gautier, Heimann, and Laudat (53)	1939		Lymphoma; Raynaud-like syndrome; data insufficient.
Gray, Greenfield, and Lederer (57)	1940		Chronic benzene poisoning; cold hemagglutination appeared only after 3 blood transfusions; data insufficient.
Jessen and Bing (78)	1940	1/33,000,000	Choledocholithasis; Banti's syndrome; uremia; data insufficient.
Tronnberg (179)	1941		2 cases of sulfapyridine treated pneumonia; one died of agranulocytosis.
Benians and Feasby (11)	1941	1/1000 1/4000	2 cases of Raynaud's syndrome with moderate anemia.
Parish and MacFarlane (144)	1941		Urticaria and angioneurotic edema not related to exposure to cold.
Reisner and Kalkstein (147)	1942		Hemolytic anemia; unusual thermal amplitude of cold hemagglutinin; erythrocyte saline fragility not increased; agglutinin persisted after splenectomy.
Rothstein and Cohn (157)	1942	1/2	Hemolytic anemia; liver damage; sulfathiazole toxicity.
Dameshek (32)	1942	1/128	Hemolytic anemia; sulfonamide toxicity.

TABLE I—*Concluded*

AUTHOR	YEAR	TITER	DIAGNOSIS AND REMARKS
Stats (170)	1943	1/256	10 days after bronchopneumonia; massive pulmonary embolus.
Stats (170)	1943	1/16	Generalized lymphosarcomatosis; agglutinin present until death (2 months).
Wiener, Oremland, Hyman and Samwick (207)	1941		Untypable pneumonia; strong rouleaux formation at 37°C.; blood transfusion without a reaction.
Stats and Bullowa (169)	1943	1/32,000	Gangrene of fingers and toes; agglutinin present.
Higgins and Roen (71)	1943		Acquired hemolytic anemia; spherocytosis; increased fragility of red blood cells; splenectomy; death.
Peterson, Ham, and Finland (146)	1943	1/10 to 1/10,000	Cases of primary atypical pneumonias treated with sulfonamides; 2 cases of acute hemolytic anemia; preliminary report.
Rosenthal and Wasserman (152)	1943	1/1028	Chronic hemolytic anemia; splenectomy; clinical cure but persistence of cold hemagglutination.
Rosenthal and Wasserman (152)	1943	1/128	Bronchopneumonia treated with sulfanilamide; blood transfusions without reaction; agglutinin not present 3 months later.
Horstmann and Tatlock (208)	1943	Up to 1/4096	27 of 40 patients with primary atypical pneumonias; two cases of acute hemolytic anemia.
Dameshek (212)	1943		2 cases of 'virus pneumonia' treated with sulfonamides; acute hemolytic anemia. Case 3 infectious mononucleosis treated with sulfadiazine; acute hemolytic anemia; cold hemagglutinin not typical.
Davidsohn (213)	1942		Raynaud's syndrome.
Helwig and Freis (214)	1943	1/5000	Acrocyanosis on exposure to cold; preceding respiratory infection may have been primary atypical pneumonia.
Turner, Nisnewitz, Jackson and Berney (215)	1943		83 cases of atypical pneumonia. The peak of elevation of the titer of cold hemagglutinins occurs between 10 to 20 days after the onset of the disease. Cold hemagglutinins in atypical pneumonia behave as specific immune antibodies.
Stratton (216)	1943		1 case of bronchiectasis; 1 case of subacute bacterial endocarditis; 1 case of carcinoma of the lung; 1 case of carcinoma of the ileum; 1 case of hemolytic anemia of moderate severity; the erythrocyte hypotonic saline fragility was increased; Raynaud's syndrome was present.

test sera, the resulting agglutination may be interpreted as isoagglutination; the conclusion will be that the blood group is AB. The serum from a patient of blood group AB should be free of isoagglutinins. However, the serum from a patient with potent cold agglutinins will agglutinate any red cells, including group O, at low temperatures and often at room temperature. It is advisable to check group AB results by test of the patient's serum for isoagglutinins or by the performance of the grouping test at 37°C.

To guard against errors in blood grouping when cold hemagglutinins of more than minimal titer are present, it is advisable to use erythrocytes washed by centrifugation with normal saline solution. With low strength cold hemagglutination, three washings at room temperature are sufficient; with higher titers, the saline solution should be warmed to 37°C., and as many as six washings may be required. If cells prepared in this manner are mixed with potent anti-A and anti-B grouping sera, using either the slide or test tube technique at 20 or 37°C., the blood grouping reaction will be dependable. If unwashed erythrocytes are to be used for grouping, the tests must be performed at 37°C. for at this temperature cold hemagglutinins (except for rare instances) are not active.

In the performance of the cross match test using the donor's erythrocytes and the patient's serum, cold hemagglutination can be prevented only by carrying out the reaction at 37°C. This is most conveniently performed in serological test tubes. Not only must the test be performed at 37°C. but it must be read at this temperature. The cooling during subsequent centrifugation may be sufficient to activate the cold hemagglutinin and vitiate the results. If the patient's serum is stored in the icebox with blood clot, a large proportion or all of the cold hemagglutinins will be absorbed from the serum, provided that the titer is not too high. In such cases cross-matching tests with the donor's erythrocytes may be carried out at room temperature.

Gross Examination of the Blood. The gross examination of the blood will often provide valuable information. When cold hemagglutination of more than minimal titer is present, hemagglutination is visible along the side of the tube into which the blood has been poured or along the barrel of the syringe used to withdraw the blood. In either case slight cooling under running tap water accentuates the phenomenon. Intense roulcaux formation in hyperglobulinemia may give a similar appearance but it is not affected by temperature. Cold hemagglutination may also be observed in hanging drop or rimmed cover slip preparations of whole blood.

Acute Infectious Diseases

Pneumonia, usually atypical pneumonia, is the most common acute febrile infectious disease in which cold hemagglutination has been observed. Staphylococcemia, scarlatina, and tonsillitis have also been recorded. Obviously the phenomenon is rare in all these conditions. Some of the patients recovered. Some of the later cases had been treated with one of the sulfonamide drugs. In all, the hemagglutination appeared during the acute phase of the disease or early in convalescence and disappeared in a relatively short time. The cold hemag-

glutinin titers in these cases varied greatly and except for one instance were below 1/500. There were no constant clinical symptoms in these cases (other than blood counting or grouping anomalies) by which the phenomenon could have been suspected. Though many of these patients were anemic, hemolytic anemia was not present, except in the sulfonamide cases. In both of the cases which we studied, cold hemagglutination was observed after pneumonia. In one, an otherwise healthy young man,[7] the phenomenon was only observed two weeks after a mild atypical bronchopneumonia (virus ?) and after the patient had developed a large pulmonary embolus. The second case was observed during the course and convalescence from pneumonia of undetermined etiology.

In the latter,[8] 20 days after the onset, cold hemagglutinins in a titer of 1/128 at 0°C. were observed when the patient was typed preparatory to blood transfusion. This 50 year old woman had been ill with fever to 103°F., cough and purulent sputum. The physical and roentgenographic signs were those of a patchy bronchopneumonia. Sputum examination revealed hemolytic (B) streptococcus and later, type 8 pneumococcus. There was no bacteremia. Sulfanilamide administration did not affect the course. Thirty-seven days after the onset, type 8 rabbit serum was given intravenously followed by a moderately severe anaphylactoid reaction. Immediately thereafter improvement set in and the patient recovered. Three indirect blood transfusions were given without any reaction. Three months after the onset, cold hemagglutinins were no longer present and the patient has remained well.

Peterson, Ham and Finland (146) have recently published a preliminary report on the frequent occurrence of cold hemagglutination during the latter part of the illness or during convalescence from primary atypical pneumonia (virus ?). Horstmann and Tatlock (208) confirmed these findings. They found elevated titers of cold hemagglutinins in eight of nine patients whose serums were tested shortly after bleeding and in only nineteen of thirty-one patients whose serums had been stored before testing. They found that the titers rise about one week after the onset of symptoms and may persist for several weeks after recovery. There was no relationship between the height of the titer and the severity of the pneumonia. However, among the few high titers were two cases of acute hemolytic anemia. They stated that the cold hemagglutinin test may be a valuable diagnostic measure in some forms of primary atypical pneumonia. These are the only systematic studies of cold hemagglutination in infectious diseases. Further work along this line is desirable.

Hematologic Disorders

Acquired Hemolytic Anemia. The entire early French school of hematologists led by Widal (190, 191) and Chauffard (23) believed that cold hemagglutination was almost pathognomonic of acquired hemolytic anemia. This is mentioned here mainly as an historical note, for it is now known that many cases of atypical non-congenital hemolytic anemia occur without cold hemagglutinins. As our

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[8] Mount Sinai Hospital #420968.

experience has broadened and as our ability to diagnose atypical hemolytic anemia has progressed, we have learned that even in this small group of cases cold hemagglutination is exceptional. Mino (123) stated that the cases described by Widal as cold hemagglutination were pseudoagglutination (rouleaux).

Reference to Table I will show that the cases of hemolytic anemia do not fall into a single category. The resistance of the erythrocytes to hypotonic sodium chloride solution varied. In some the fragility was normal; in others it was either increased or decreased. Macrocytosis was mentioned in some reports. In others the erythrocytes were presumably of normal size; in those in which increased fragility was present, spherocytosis may have occurred. Thrombocytopenia and a hemorrhagic diathesis were noted in certain cases. In some of the cases of hemolytic anemia the agglutinins disappeared spontaneously as the blood destruction ceased; in others they disappeared after splenectomy and in still others they persisted with little change in titer after splenectomy and cure of the anemia.

Enough cases of favorable response to splenectomy in acquired hemolytic anemia with cold hemagglutination have been recorded to establish this operative procedure as rational and effective therapy for this disease. However, the occasional spontaneous cessation of hemolysis and disappearance of the cold hemagglutinins indicate that supportive therapy should be given an adequate trial. A case of hemolytic anemia that we observed is described under the heading of pregnancy (see below). Nakamura (133) found no change in the titer of cold hemagglutinins in normal rabbits after splenectomy.

A pathogenetic relationship between the cold hemagglutination and the hemolytic anemia in these cases is questionable. The recent revival of interest in the hemolysin theory of hemolytic anemia (29, 30, 31) brings to mind the possibility that the antibody in the cases described might in some way cause increased destruction of erythrocytes. It is not uncommon to observe hemolytic anemias identical in all respects, except for the absence of cold hemagglutination, with the cases showing cold hemagglutination. In some such anemias the hemolytic process is terminated by splenectomy. In some of the cases with cold hemagglutination, the agglutination persisted unchanged after disappearance of the hemolytic anemia. In very few of the cases (see below) has a lytic action of the cold hemagglutinin been demonstrated. In none of these nor in any of the acute hemolytic anemias following sulfonamide administration (see below) has actual activation of the agglutinin in vivo by cold been demonstrated. Some of these cases and many of the acute cases were actually febrile at the time of the hemolytic episode. The cold hemagglutinin has no demonstrable action at body (37°C.) or at higher temperatures. Tho many other cases in Table I in which similar or higher titers of cold hemagglutinins were recorded did not have hemolytic phenomena. In some of these cases activation of the agglutinin was demonstrated by exposure of the patient to cold; transient hemolysis was the result. The accumulated evidence favors the view that the cold hemagglutination in these cases of hemolytic anemia is not the cause but may be the result of the anemia.

This subject cannot be closed without reference to reported cases (146, 208, personal observations) of acute hemolytic anemia with high titers of cold hemagglutinins occurring during the course of primary atypical pneumonia. Similar or higher cold hemagglutinin titers in this type of pneumonia may occur without the superimposition of hemolytic anemia. Hemoglobinemia may or may not be present. In the case[9] which we observed, recovery followed several uneventful blood transfusions. In this case the hypotonic fragility of the erythrocytes was normal, the urobilinogen excretion was high and spontaneous hemoglobinemia was not observed. Despite this, hemoglobinemia was produced by immersion of an extremity in cold water and the mechanism of hemolysis could be reproduced in vitro (see below). Pari passu with the subsidence of the hemolytic phase, the cold hemagglutinin titer fell and the hemolytic phenomena could no longer be demonstrated. Most, if not all of these cases, had received sulfonamides several days before the onset of the acute hemolytic anemia. The exact causal relationship between the pneumonia, the cold hemagglutinin, the sulfonamide and the hemolytic anemia in these cases is not clear. Because of the parallelism, however, between the titer and the hemolysis, there is reason to associate the hemagglutinin and the hemolysis pathogenetically.

Shilling (162) observed transient autohemagglutination in experimental anemias produced by phenylhydrazine. Rous and Robertson (151, 158) reported that a strong cold hemagglutinin was observed in one rabbit after repeated withdrawals of blood. Nakamura (133) was not able to confirm either of these findings except that in one out of 7 rabbits a rise in titer from 1/8 to 1/512 occurred in the course of phenylhydrazine-induced anemia (also see Matsuda (113)).

Acute Hemolytic Anemia Due to Sulfonamides. A sufficient number of cases of acute hemolytic anemia due to sulfonamide administration have been observed to allow one to state confidently that cold hemagglutination is not a common finding in this condition. Even if cold hemagglutinins were not looked for in some of these cases, there certainly was no difficulty in performing red blood counts or blood groupings. If cold hemagglutinins of more than minimal potency had been present, some abnormality in either of these tests would have been observed. In the cases that we have seen, cold hemagglutinins of more than minimal titer could not be demonstrated. It is, therefore, not likely that a pathogenetic relationship between cold hemagglutination and such a hemolytic anemia can be established. The titers of cold hemagglutinins are not mentioned in all of the cases of Table I, but from the descriptions they can be assumed to have been low. Low titer cold hemagglutinins may be observed in normal individuals. The cold hemagglutinins in these cases were transient. In some the frequency of blood counts, blood groupings, and blood transfusions may have served to focus attention upon a low titer cold hemagglutinin that would not otherwise have been noticed.

Hemoglobinuria Not Associated with Exposure to Cold. Hemoglobinuria is

[9] Mount Sinai Hospital #503906.

now recognized to be the result of a level of hemoglobinemia in excess of the renal threshold for this pigment. Hemoglobinemia follows intravascular hemolysis. The question, then, of the relationship between hemoglobinuria not associated with exposure to cold and a cold hemagglutinin which may be present is essentially identical with that of the relationship between the agglutinin and acute hemolytic anemia. This has been discussed above. In further support of the thesis that the action of the agglutinin is not significant in these regards is the fact that hemoglobinuria in the absence of cold hemagglutination is common in acute hemolytic anemia. While dual or multiple mechanisms for hemolytic anemia and hemoglobinuria (restricted to the type of hemolytic anemia discussed here) may exist, the facts at our disposal do not favor such an hypothesis. Cold hemagglutination and hemoglobinuria not related to exposure to cold are reported in 5, 32, 58, 71, 147, 157, 172, 184.

In the Marchiafava-Micheli variety of hemoglobinuria (chronic hemolytic anemia with paroxysmal nocturnal hemoglobinuria and perpetual hemosiderinuria), we have not observed cold hemagglutination. The erythrocyte hemolysis by dilute acid (Ham (62)) in this disease does not occur in cold hemagglutination (146 (Personal observations)).

Paroxysmal Cold Hemoglobinuria. *Introduction.* In all these cases a direct relationship between cold and the hemoglobinuria was easily obtained in the history. In several patients the hemoglobinuria or hemoglobinemia was reproduced by exposure of the patient to the cold. In those in which this was not obtained, the cold exposure may not have been marked or prolonged. There are 10 reported cases in this category (1, 65, 87, 115, 121, 155, 160, 169, 172). Only 3 cases (87, 121) did not have a Raynaud syndrome; in two gangrene occurred (115, 169).

The observation of paroxysmal cold hemoglobinuria in these cases has raised the question of the action of cold in hemoglobinurias in general. The close relationship between low temperatures and the paroxysmal hemoglobinuria of syphilis so completely studied by Donath and Landsteiner and others has clarified this clinical entity. In our subsequent discussions we shall endeavor to demonstrate that paroxysmal hemoglobinuria following exposure to cold also occurs in cold hemagglutination and that this entity, though rare, is distinct from the syphilitic cases. Low temperatures do not accelerate hemolysis in other hemolytic syndromes or hemoglobinurias. However, with regard to blackwater fever, there is some difference of opinion for it is stated that exposure of such patients to cold may precipitate hemoglobinuria. The majority and particularly the more recent (154) writers on this subject deny any relationship. Drieux (203) stated that cold is a factor in the precipitation of attacks of equine myoglobinuria. In this condition, however, the action is on the muscles, not on antibodies or the blood.

Mechanism. A recently observed case (169) reported by Stats and Bullowa of paroxysmal cold hemoglobinuria associated with a cold hemagglutinin demonstrated the mechanism of hemoglobinuria.

Previous investigators (108) have elucidated the mechanism of hemoglobinemia

and hemoglobinuria in syphilitic cold-warm hemolysin and positive Donath-Landsteiner test) cold paroxysmal hemoglobinuria by immersing the extremities (Rosenbach) or the constricted finger (Ehrlich) in cold water.

Using precautions to prevent the occurrence of hemolysis during the withdrawal or centrifugation of the blood, venous blood was drawn from each arm; the right forearm was then placed in ice cold water. After ten minutes venous blood was drawn from the left arm. After twenty-five minutes the right arm was removed from the cold. Venous blood was immediately drawn simultaneously from each arm. Both arms were then left at room temperature and, at appropriate intervals during the subsequent one and one-half hours, additional blood samples were taken from each arm at the same time. After centrifugation the supernatant plasmas of all the specimens were examined and subjected to quantitative analysis for hemoglobin pigment by a modification of the Bing and Baker (15) benzidine method. Samples containing hemoglobin were examined by the Hardy recording spectrophotometer.[10] Urine passed before and at the conclusion of the experiment was examined for hemoglobin by the usual benzidine technique. The figures 1 and 2 summarize the findings. The hemoglobinemic specimens of plasma were examined with the acetone icterus index method for bile pigment. 2.5 units were found in each specimen. There was no evidence of bilirubin formation.

Hemolysis occurred only in the cold and was therefore a local phenomenon. The fact that the only detectable pigment was oxyhemoglobin indicated that simple hemolysis without alteration of the normal red cell pigment occurred. As soon as the arm became warm the hemolysis ceased, illustrating the strict dependence of the activity of the reaction upon temperature. The absence of systemic hemoglobinemia (blood from the left arm) indicated, again, that hemolysis was a local phenomenon and supported the conclusions mentioned above, for the blood flow to the cold arm was undoubtedly meagre. By dilution with the blood of the systemic circulation, the intense local hemoglobinemia was greatly reduced. The mechanisms existing for the removal of free circulating hemoglobin rapidly removed this pigment from the plasma. The absence of hemoglobinuria followed the absence of systemic hemoglobinemia. This experiment was carried out several times without complications. There was no systemic reaction, no chill or fever, and no fall in blood pressure. Prolonged venous stasis did not produce more hemoglobinemia (at room temperature) in this patient than in a control.

Differentiation From Syphilitic Cold Paroxysmal Hemoglobinuria (Donath-Landsteiner). There does not seem to be any relationship between cold hemagglutination, even when associated with hemoglobinuria, and paroxysmal cold hemoglobinuria of the syphilitic type. The only similarity is the importance of cold in activating the antibody.

Donath-Landsteiner Test and Syphilis: In the latter cases the Donath-Landsteiner and Wassermann reactions (92 per cent of cases) (73) are almost always positive; in the former they are almost always negative; few reports

[10] Performed at the Electrical Testing Laboratories, Inc., N. Y. C.

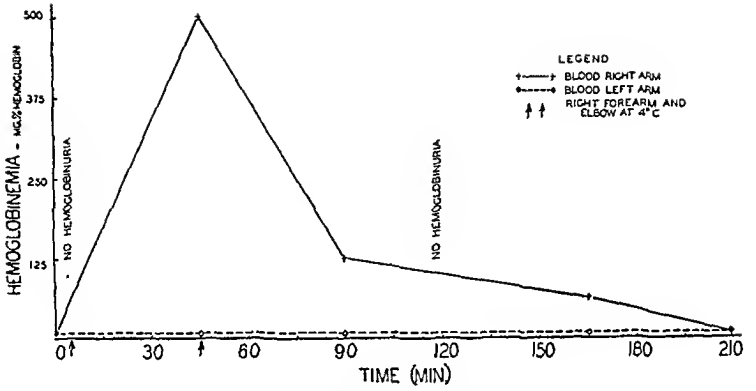

FIG. 1. UNILATERAL HEMOGLOBINEMIA PRODUCED BY EXPOSURE OF FOREARM TO COLD
Reproduced by courtesy of the Editors of the Archives of Internal Medicine (169)

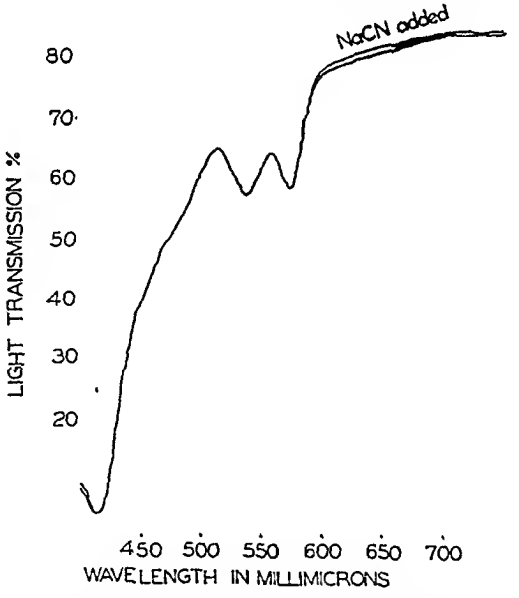

FIG. 2. SPECTROPHOTOMETRIC CURVE OF HEMOGLOBINEMIC PLASMA
Reproduced by courtesy of the Editors of the Archives of Internal Medicine (169)

mention positive Donath-Landsteiner (155) or Wassermann (27, 78, 104, 120, 126, 153, 157, 200) reactions with cold hemagglutination. The conclusion is warranted that the antibody in the two conditions is fundamentally different. The Donath-Landsteiner test is as specific as the most sensitive serological test and is positive only in syphilitic cold hemoglobinuria or in a restricted number of late syphilitics without hemoglobinuria. The one case (155) of cold hemagglutination with a positive Donath-Landsteiner test is questionable because in the same article the test is recorded negative in two places and positive in one. It is not possible to formulate a conclusion on the relationship of cold hemagglutination to complement fixation or precipitation tests for syphilis. In the great majority of reports of cold hemagglutination in disease there was no clinical or serological evidence of syphilis. The autopsies of patients with cold hemagglutination during life, including two personally observed instances, did not reveal tissue changes of syphilis (see under trypanosomiasis). There is no question that there is no obligate relationship between cold hemagglutination and syphilis. When these conditions occur in the same patient the association may be coincidental.

It could be maintained that the Donath-Landsteiner test or other in vitro tests might be positive in cases of cold hemagglutination if they were sufficiently sensitive. While this may be true, there is no evidence to support it. Further discussions of this question are given in the section "Laboratory Aspects of Cold Hemagglutination" under 'the hemolytic mechanism in cold hemagglutination.'

Skin Reaction to Cold: The dermal sensitivity to cold observed in the syphilitic cases is of an "allergic" nature (66, 108) and is related to so-called physical cold allergy (102). Urticaria and angioneurotic edema are often present. Except for occasional case reports, the skin reaction to cold in patients exhibiting cold hemagglutination is of an ischemic nature due to obstruction of the circulation by agglutinated cells. Of the 4 case reports of urticaria and angioneurotic edema with cold hemagglutination, 2 are questionable. In none did the angioneurotic edema closely follow exposure to cold. In a case of cold hemagglutination, Roth (155) reported that application of ice to the skin did not give rise to a wheal, flare, or urticaria. We have confirmed this in 2 cases.

Systemic Reactions to Cold: Upon general exposure to cold the syphilitic cases often develop a systemic reaction; this may be lacking in the other group. In our recently studied case (169) this seemed to be true, for the systemic reaction on exposure to cold, at least in the experiment in which the forearm was immersed in cold water, was not present. However, the patient stated that epigastric and lumbar pain occurred as a rule with his paroxysms of hemoglobinuria following exposure to environmental cold. No fever or undue weakness accompanied these episodes. The term 'autoanaphylaxis' has been employed by Micheli (116) and 'hemoclasic crisis' by Widal, Abrami, and Brissaud (192) to describe the phenomena in luetic paroxysmal hemoglobinuria after exposure to cold. These terms can be employed to describe the clinical state accompanying any severe episode of hemoglobinemia and hemoglobinuria. Hemolytic transfusion reactions as a result of incompatible blood, severe acute hemolytic anemia, and the

reaction to sudden hemolysis in Marchiafava-Micheli (52) disease fall into this group. The data in the recorded cases of cold hemagglutination with hemoglobinuria are not detailed enough to permit a generalization with regard to the presence or absence of a severe systemic reaction on exposure to cold. If the hemolysis were marked, the same general symptoms and signs (chill, fever, abdominal, back and extremity pain, headache, fall in blood pressure, diminution in the coagulability of the blood, and leukopenia) might occur. However in 14 experimentally induced episodes of hemoglobinuria in a patient with a potent cold hemagglutinin, Salén (160) did not observe any evidence of a hemoclasic crisis. He called special attention to the difference, in this regard, from luetic cold paroxysmal hemoglobinuria.

Very little attention has been paid to the fate of the stroma of the erythrocytes in a consideration of systemic reactions to hemolysis in vivo. It is probable that the pathogenesis of many of the results of intravascular hemolysis are related to the action of the particular mode of hemolysis on the stromatic lipoids and proteins of the red blood cells (except for the effect of hemoglobin upon the kidneys). This phase of hemolytic reactions is worthy of detailed investigation (46).

Hemagglutination and Hemolysis: Mattirolo and Tedeschi in 1903 (114) were the first to record data on the occurrence of hemagglutination in cases of syphilitic cold hemoglobinuria. They described 2 cases in which slight agglutination of homologous erythrocytes was observed at 37°C. They did not perform experiments in the cold. The significance of this observation cannot be stated. In 1909 Moro, Noda, and Benjamin (131) mentioned the occurrence of hemagglutination during the warming of previously chilled blood in a similar case. Foix and Salin (47) in an incomplete report mentioned the presence of autoagglutination of the red blood cells in a case of luetic paroxysmal hemoglobinuria. The effect of temperature was not recorded. Two years later Moss (132) stated that hemagglutination of significant degree does not occur in the luetic cases even in the cold. Lüdke (107) has been quoted to have observed autoagglutination in paroxysmal hemoglobinuria. On the contrary, however, he stated that in 2 cases of congenital hemolytic icterus and in one case of acquired hemolytic icterus autoagglutination was observed. Mino (125) in 1924 was the first to study this question intensively. In a typical case in which the Donath-Landsteiner reaction was positive, he showed that (1) the serum of the patient neither agglutinated nor hemolyzed the homologous red blood cells at 0, 14–17, or 37°C., (2) the serum agglutinated and hemolyzed the cells if after chilling at 0°C. the temperature was increased to 37°C., (3) the diluted serum only agglutinated the red cells during the procedure of chilling and subsequent warming, (4) the agglutinating power of the serum could be exhausted by absorption with erythrocytes. He quoted Micheli as follows: "Autoagglutinins are constantly accompanying the autohemolysin in paroxysmal hemoglobinuria and are activated by cooling and only by cooling." Montanari (127) substantiated the findings of Mino in this regard, recording a case in which a hemagglutinin titer of ¼ was found. He stated that when the temperature of a mixture of cells and

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serum was gradually increased from 4 to 15–20°C., agglutination was observed. Brulé, Hillemand, and Gaube (21) did not find autohemagglutination in their first case. Amzel and Hirschfeld (3) mentioned a case with a positive Donath-Landsteiner reaction in which cold hemagglutination from 0 to 3°C. was observed.

These observations are unique in the vast literature of syphilitic cold paroxysmal hemoglobinuria and have not received much attention because of the greater importance of the demonstration of hemolysis by chilling followed by warming. However, they are of significance in a consideration of the question of cold hemagglutination, for they demonstrate that the action of the antibody in causing agglutination in the syphilitic disease is fundamentally different from the antibody action in cold hemagglutination. In the latter condition the maximum hemagglutination is observed at about 4°C. As the temperature is increased the agglutination disappears. Cold hemagglutination of low titer is a constant finding in normal individuals. It presumably should also occur in syphilitic cold hemoglobinuria but obviously could be independent of the disease.

A type of hemolysis dependent upon a high titer of the cold hemagglutinins, a high concentration of erythrocytes and mechanical trauma can be demonstrated in vitro in the cold in certain cases with cold hemagglutinins. This phenomenon is described more completely under 'The hemolytic mechanism in cold hemagglutination' and in a separate communication (206). In vitro hemolysis has previously been described in 11, 20, 33, 160, 172 and 200. Both antibodies combine with their respective antigens (erythrocytes) in the cold but the action in the luetic cases can be demonstrated only in the presence of complement when the temperature is increased to 37°C.; hemolysis occurs at this temperature. In the cold hemagglutination cases rapid dissociation of agglutinin and antigen occurs when the temperature is brought up to 37°C. and the agglutination breaks up; hemolysis is not observed. As will be discussed presently, cold hemagglutinins occur in most, if not all, normal human blood sera whereas cold hemolysins are found in cases of syphilis with or without cold hemoglobinuria. Landsteiner (93) stated that the autohemolysin in paroxysmal hemoglobinuria (syphilis and positive Donath-Landsteiner test) is specific for human blood. The hemolysin acts only on human erythrocytes. The cold hemagglutinin has no such specificity and acts on the erythrocytes of many species.

The difference in test tube reactions between the antibodies in cold hemagglutination and those in the cold-warm hemolysis of the luetic type is of basic importance. Hemolysis in vitro by antibodies is carried out only by participation of complement in the reaction (excepting the participation of trauma). If the complement is not present, hemolysis does not result. For some as yet unknown reason, certain antibodies take up complement when they combine with their antigens and thus have a lytic action, while other very similar antibodies when combining with the same antigen, do not. This has been studied extensively by Horsfall and Goodner (74) in the reactions of rabbit and horse antipneumococcic antibodies with the specific capsular carbohydrates of pneumococci. The cold hemagglutinin may be thought of as similar to human or horse antipneumococcic antibody in that complement is not used in the reactions with

specific antigens (the evidence for this is presented below); on the other hand, the hemolysin of syphilitic cold hemoglobinuria is similar to the rabbit antipneumococcic antibody in that complement is used. The hemolysis in the Donath-Landsteiner test depends upon the fact that complement enters into the reaction between the red blood cells and the antibodies. The lack of hemolysis in cold hemagglutination is due to the absence of complement in the red blood cell-agglutinin complex (excepting the participation of trauma).

Association With Other Conditions—Raynaud's Syndrome: Lewis (101) and MacKenzie (108) discussed the variegated peripheral circulatory phenomena which may occur in syphilitic paroxysmal cold hemoglobinuria and mentioned the unusual occurrence of gangrene of the extremities in such cases. Raynaud's syndrome either complete or as "formes frustes" is common. Similarly in cold hemagglutination with paroxysmal cold hemoglobinuria tingling of the fingers, toes, tip of the nose or lobes of the ears, and cyanosis, coldness or pallor upon exposure to cold have been mentioned many times. There are 7 reports of functional vasomotor disturbances of the extremities associated with paroxysmal cold hemoglobinuria and cold hemagglutination (1, 65, 155, 160, 172) and 2 reports of gangrene (115, 169). The pathogenesis of the Raynaud phenomena and the gangrene in these cases will be discussed below.

Other Diseases: While syphilitic cold paroxysmal hemoglobinuria occurs in individuals with manifest evidence of syphilis clinically, or in apparently normal (but late syphilitic) individuals (serologically positive for syphilis), most of the cases of cold hemagglutination paroxysmal cold hemoglobinuria have been reported unassociated with other disease. One case had lymphatic leukemia (1) and another hemolytic anemia (160). All of the cases of cold hemagglutination with hemoglobinuria not associated with cold occurred in patients with hemolytic anemia.

Summary:

Leukemia, Pernicious Anemia, Lymphoblastoma and Thrombocytopenic Purpura. Reference to Table I will show the relatively few cases of these conditions associated with cold hemagglutination. The case that we observed was a 16 year old female[11] who died of diffuse lymphosarcomatosis. During life she had a cold hemagglutinin titer of 1/16; the Wassermann reaction was negative; there was no hemolytic process; Raynaud's syndrome was absent; several blood transfusions were given without any reaction. The patient died of peritonitis due to perforation of an intestinal ulcer.

Tropical Diseases

Trypanosomiasis. African trypanosomiasis was one of the first diseases in which cold hemagglutination was found (24, 40, 79, 111, 112, 178, 201). It remains as the only disease except atypical pneumonia in which this phenomenon occurs with regularity. However, not all cases show the phenomenon. Yorke's paper (201), which is one of the earliest clear discussions of hemagglutination, mentioned that monkeys, goats, dogs, rabbits, guinea pigs, and rats devel-

[11] Mount Sinai Hospital #497192.

oped cold hemagglutinins when infected experimentally with trypanosomes. He was not able to ascertain why the infestation was associated with cold hemagglutinins but did observe a disappearance of the antibodies upon recovery from the disease. He also presented valuable data on the occurrence of agglutination in normal animal blood.

A reaction known as the "red cell adhesion" reaction has been described in African trypanosomiasis (209). This reaction manifest at room temperature or at 37°C. occurs when a suspension of trypanosomes, complement, primate erythrocytes and serum from certain patients with trypanosomiasis are mixed. The red blood cells (also blood platelets and certain colloidal dyes) become adherent to the trypanosomes. When the reaction is marked the parasite may

TABLE II

	COLD HEMAGGLUTINATION	COLD-WARM HEMOLYSIS, DONATH-LANDSTEINER, SYPHILIS
Occurrence	Most normal individuals	Practically only in some cases of late syphilis
Syphilis	Rare	Almost invariable
Donath-Landsteiner Reaction	Negative	*Positive*
Hemagglutination in cold (4°C.)	Marked	Slight or absent
Complement absorption in cold	None	Present
Specificity of antibody	Slight	Marked
Skin reaction to cold	Ischemia (more observations required)	Urticaria, angioneurotic edema.
Systemic reaction to cold	Not certain; may be lacking	Usually present — antoanaphylaxis
Raynaud's syndrome	Present	Present
Gangrene of acra	Present	Present
Occurrence in absence of hemoglobinuria	Yes in trypanosomiasis, hemolytic anemia, cirrhosis of liver, etc.	Yes in late syphilis (especially paresis)
Hemolysis in the cold with trauma to erythrocytes	Marked with high titers	Probably none

become completely covered with red cells, the so-called caddis form, and resemble a mass of agglutinated erythrocytes. This phenomenon is unrelated to cold hemagglutination. Duke and Wallace (209) stated: "autoagglutination, per se, does not necessarily cause the adhesion of red cells to trypanosomes."

It is well known that falsely positive Wassermann reactions are common in trypanosomiasis. The fact that cold hemagglutination also occurs in this disease raises the question that some of the positive Wassermann reactions with cold hemagglutination in other diseases may likewise be falsely positive. Studies on the occurrence of cold hemagglutination in non-African trypanosomiasis are not available.

Relapsing Fever. Laboratory animal infections with borrelia recurrentis

(relapsing fever) were often accompanied by cold hemagglutination (134). This phenomenon is not mentioned in recent texts (174) of tropical diseases in association with relapsing fever in man.

PIROPLASMOSIS. There is one reference to the occurrence of cold hemagglutination in young dogs infected with piroplasmosis. The agglutination was not as marked as in experimental infections with trypanosomes (135).

MALARIA. The question of hemagglutination in malaria is an old one, having occupied the attention of numerous writers for many years. The details of this are beyond the scope of the present report and the reader should refer to the sources for further information.

There is one case report in the literature in which cold hemagglutination was observed in a patient with chronic malaria (8). The phenomenon disappeared after splenectomy in that case. It will be observed in Table I that in a few cases malaria had been present—in most of these the infection occurred years before the detection of cold hemagglutination and was inactive. In numerous places in the literature the phenomenon is said to occur with malaria. There is no reference to this in Strong's recent text (174). Hirshfeld (68) could not demonstrate "autoagglutination" in a large series of cases of malaria. The hemagglutination which has been described is probably rouleaux formation (14) and this and the anemia are responsible for the rapid erythrocyte sedimentation rate in malaria. Lack (88) has described intravascular agglutination in malarial infected canaries by use of a quartz rod micro-illuminator. He did not state whether this was true agglutination or rouleaux formation. Schuffner and Esseveld (164) observed true hemagglutination in a single blood film from a patient with malignant malaria. Eaton (210) concluded his study of agglutination reactions in malaria by stating "that immune plasmodium knowlesi monkey serum agglutinates mature intracellular or extracellular parasites but does not agglutinate unparasitized red cells or red cells containing immature parasites."

The situation may be summarized as follows:

1. Cold hemagglutination has not been conclusively demonstrated regularly in malaria or blackwater fever (22).

2. A type of agglomeration of erythrocytes has been described especially in blackwater fever or in severe malarial infections

   a. on the microscopic slide (28)

   b. in vivo in experimental avian and monkey (quoted by 88) infections

   c. at autopsy in the internal organs in human cases. The agglomerated erythrocytes are usually parasitized (42, 109, 205).

3. The sedimentation rate in active malaria is increased due to rouleaux formation. The blood globulin is elevated at this time.

4. Autolysins or lysins for the same blood group erythrocytes have not been observed by most authors in blackwater fever (154).

5. The agglomeration of erythrocytes at autopsy in humans and in vivo in animals may be due either to antiplasmodia agglutinins or to rouleaux formation.

It is likely that the solution of the problem of hemagglutination will shed light on numerous phases of the pathology, clinical manifestations, immunity, and pathogenesis of malaria and blackwater fever.

Cirrhosis of the Liver

We have previously commented on the publications of Hayem (67), Reitmann (148) and Klein (83) in which there was a question of whether agglutination, rouleaux formation or some other abnormality was present. All these were cases of cirrhosis of the liver. In the subsequent publications cirrhosis of the liver is frequently recorded in association with cold hemagglutination. While in some cases the type of cirrhosis of the liver is not mentioned, many are of the type denoted as "hypertrophic" or "infective." Hanot's cirrhosis has also been mentioned in this regard. So far as can be determined, atrophic Laennec cirrhosis of the liver is not often associated with cold hemagglutination. In some of the cases of cirrhosis, the patients had been imbibers of alcoholic beverages.

Raynaud's Syndrome (75)

Introduction. There are 12 (1, 11, 34, 53, 65, 76, 77, 155, 160, 166, 172) instances in which cold hemagglutination occurred in patients who also had functional circulatory insufficiency in the extremities. While not all of these are complete enough to warrant inclusion under the heading of classical Raynaud's syndrome, they are grouped here because they all have a similar pathogenesis. It has even been proposed that cold hemagglutination may be the cause of the ischemia in the large majority of cases of Raynaud's syndrome (76, 77, 98). This is highly questionable since serological study of 4 cases of Raynaud's syndrome by the authors did not reveal cold hemagglutination.

The hypothesis to be discussed below regarding the mechanism of the Raynaud syndrome in cases of cold hemagglutination is that on exposure of the body to cold two events transpire. In the first place vasoconstriction of peripheral blood vessels and a fall in temperature in the fingers and toes is a normal phenomenon (100). This process reduces the blood flow to the areas in question. If at the same time marked hemagglutination occurs within these narrowed vessels or in the capillaries of the regions, further retardation or even cessation of blood flow might occur. In a very short time the color and sensory changes of Raynaud's syndrome supervene. With warming of the body, vasodilatation and disruption of the hemagglutination would be followed by a return of blood flow. Such a process could occur repeatedly on exposure to cold.

Certain studies carried out in a recently observed case (169) of cold hemagglutination and Raynaud's syndrome will serve here to confirm the above hypothesis.

For hemagglutination to be an important pathogenetic factor, it is naturally important to obtain data on the rapidity of the reaction and on its reversibility.

Rapidity and Reversibility of Cold Hemagglutination. Complete and even dispersion of the red cells was observed when a mixture of erythrocytes and serum (proportions of normal blood) from a patient with potent cold hemagglutination was placed in a tube at 30°C. or higher, i.e., at or slightly below the normal temperature of the tips of the fingers, the lobes of the ears, or the tip of the nose. When the tube was plunged into ice water, hemagglutination was visible grossly in one-half minute and in 2 minutes massive total agglutination was so marked that the red cell mass resembled a gel. This state persisted so

long as the temperature was maintained below 20°C. If the iced tube and its contents were suddenly plunged into water at a temperature of 37°C., the massive agglutination broke up in less than one minute and shortly thereafter complete dispersion of the cells occurred. This procedure of causing agglutination by cold and resuspension by warmth was repeated many times with the same specimen without change or apparent cell injury.

It can be readily appreciated that these serological characteristics of cold hemagglutination, especially the rapidity of change with temperature alterations, ideally suit this phenomenon to a Raynaud symptom complex.

Hemagglutination Intra Vitam. In the same case mentioned above (169) intra vitam agglutination of the erythrocytes exposed to cold was also demonstrated. This experiment was suggested by similar ones in cases of cold hemagglutination performed by Iwai and Nin (76, 77) and Jessen and Bing (78) who were able to observe hemagglutination in conjunctival capillaries. Nakamura (133) applied ice to the ear of a rabbit in which a potent cold hemagglutinin had been found. He described macroscopic fragmentation of the blood columns due to hemagglutination. Fahraeus (45) had previously described the intra vitam occurrence of rouleaux formation in the folds of the finger nails, veins of the forearm, and retinal vessels in cases with a high degree of rouleaux. Wintrobe and Buell (199) reported the occurrence of Raynaud's syndrome in a patient with multiple myeloma and marked rouleaux formation. Lewin (99) and Debenedetti (36) mentioned the occurrence of erythrocyte agglutination in the tissue sections from necropsy of patients who had shown cold agglutination during life. In 2 recent cases we were unable to confirm this last finding. (Mount Sinai Hospital #498441 and #497192.)

To demonstrate hemagglutination intra vitam we[12] irrigated the conjunctival sac of a patient with 100 cc. of iced isotonic saline solution and then immediately viewed the vessels of the bulbar conjunctiva with the binocular corneal microscope. Marked segmentation or discontinuity of the vascular columns was visible; between succeeding masses clear plasma was seen. As observation continued the segments moved slowly and gradually broke up under the influence of environmental (22°C.) temperature. This procedure was carried out several times in each eye without complications. A normal control eye subjected to the same procedure revealed immediate blanching followed by a reactive hyperemia.

The physiologic basis for the occurrence of Raynaud's syndrome in patients with cold hemagglutination is clearly demonstrated by these experiments. Marked and rapid hemagglutination due to cold obstructs the capillary circulation. The circulation is resumed following an increase in temperature and the changes disappear rapidly.

In a previous publication (169) (Stats and Bullowa) we have discussed the differential diagnosis of transient circulatory insufficiency of the extremities despite the presence of adequate pulsations of the palpable arteries.

[12] Performed with the assistance of Dr. Arthur Minsky, ophthalmologist, Harlem Hospital, Department of Hospitals, N. Y. C.

Pregnancy

In 3 cases, Kligler (85), Stewart and Harvey (171), and in a personally observed case,[13] the phenomenon of cold hemagglutination was observed in a pregnant woman. In the case of Kligler, the patient was anemic, presumably as a result of bleeding hemorrhoids. The fetus was delivered alive, full term; its blood was free of cold hemagglutinin. Stewart and Harvey's case had a hemoglobin as low as 18 per cent. The cause of the anemia was not entirely clear. A full term normal fetus was delivered. The cold hemagglutinin persisted in the mother after delivery. Our case warrants a more detailed discussion. This woman had delivered two full-term still-birth fetuses before coming under observation. She was found to have a severe hemolytic anemia which had apparently started toward the termination of her last pregnancy. A high titer cold hemagglutinin was present. The hypotonic erythrocyte fragility was normal. The hemolytic process was terminated by splenectomy and the hemolytic anemia cured. Microscopic examination of the spleen revealed the same "arterial hyperemia" seen in other cases of hemolytic anemia. The cold hemagglutinins persisted without change in titer. Numerous blood transfusions were given without reactions. A third pregnancy was uneventful and the fetus was delivered at term. Three days after delivery cold hemagglutinins were not present in the infant's blood. This child died at the age of 6 months after a series of gastro-intestinal upsets and skin infections. Both the mother and the child had an unclassified hereditary ectodermal dysplasia.

Venous Thrombosis and Pulmonary Embolism

There is a considerable literature on the association of cold hemagglutination and bland venous thrombosis and pulmonary embolism. Neuda (138) believed that hemagglutination was the forerunner of venous thrombosis. Inherent in the mechanism of hemagglutination he demonstrated a lytic or dissolving quality to the serum (139). The hemagglutinin which caused the thrombosis could at a subsequent time be responsible for embolization by a destructive action upon the clot. He stated that spontaneous thrombo-embolic processes are prevalent among the descendants of syphilitic and carcinomatous individuals. He also stated that thrombosis is common in anemic patients and that hemagglutination is a frequent finding. He advised daily injections of liver extract as a prophylaxis and therapy of thrombo-embolic states. He did not present any case reports or clinical data supporting his contentions. Other authors (185, 204) were not able to confirm some of his findings. A complete reinvestigation of the relationship between venous thrombosis, pulmonary embolism, and cold hemagglutination should be performed.

We have added one case report to the only similar report in the literature (see Table I). Our patient,[14] a young man of 18 years previously well and not luetic, was convalescing from a mild atypical bronchopneumonia when he suddenly developed a pulmonary infarct. Several days later he sustained a

[13] Mount Sinai Hospital #416010.

[14] Mount Sinai Hospital #498441.

fatal pulmonary embolus. The titer of cold hemagglutinins in this case was 1/256 at 4°C.

Peterson, Ham, and Finland (146) in their study of primary atypical pneumonia in which cold hemagglutinins were found mentioned cases which developed phlebothrombosis and pulmonary emboli. These cases are probably similar to the one we have observed.

Fahraeus (45) has reviewed the literature on the question of the relationship between rouleaux formation and thrombus formation. He has presented evidence for the thesis that marked rouleaux formation or decreased suspension stability of the blood may be of importance in initiating thrombosis in pregnancy, the puerperium, and in acute infectious diseases when the sedimentation rate of the blood is increased.

Gangrene of the Extremities

There are only 2 cases (115, 169) in the literature, both men who formerly worked with aluminum, in which gangrene of the fingers or toes brought on by moderate cold was associated with cold hemagglutinins. For a complete review of this phase of the subject of cold hemagglutination the reader is referred to the paper of Stats and Bullowa (169).

The mechanism of gangrene in these cases is the same as the mechanism of the Raynaud syndrome previously described in this communication.

GENERAL FEATURES OF COLD HEMAGGLUTINATION

Occurrence in Normal Animals and Human Beings

Landsteiner (92) was the first to demonstrate the occurrence of cold hemagglutinins in the serum of normal guinea pigs, chickens, horses, dogs, rabbits, and cattle. Wheeler (188) made an exhaustive study of the cold hemagglutinins in normal rabbit sera. Ottenberg and Thalhimer (142) described the phenomenon in cats. Walther (183) recorded autoagglutination in sheep, monkeys, donkeys, and goats. Yu (202) presented detailed data on the serological characteristics of cold hemagglutinins in normal animal sera. Amzel and Hirszfeld (3) and Kettel (80, 81, 82) have performed exhaustive investigations on the occurrence of cold hemagglutinins in normal human sera. The former were able to demonstrate such agglutinins in 47 per cent of 238 sera. With improvements in technique the latter demonstrated the antibodies in 95 per cent of 600 sera and stated that cold hemagglutinins can probably be found in all normal human sera. There are no recorded studies of the incidence of cold hemagglutinins or of their properties in perfectly normal healthy persons. The material of Amzel and Hirszfeld was from routine bloods sent to the laboratory for Wassermann tests. Kettel's material consisted of 600 persons most of whom were hospitalized for chronic diseases. A few bloods from healthy individuals or acutely ill patients were included in the study.

Kettel emphasized the importance of allowing blood to clot at 37°C. so that none of the cold agglutinins were absorbed by the homologous erythrocytes. He could not find any relationship between the anti-A and anti-B isoagglutinin

titers and the titer of cold hemagglutinins. There is no relationship between the Hübener-Thomsen agglutinin titer and either the isoagglutinin or cold hemagglutinin titers (48). Montanari (128) did not find any relationship between agglutinin titers for various bacteria and cold hemagglutinins.

Cold agglutinins were found by Kettel in all blood groups but tended to occur in highest titer in blood group B. In the performance of exact titer tests of cold agglutination, he found in general an irregular variation in the sensitivity of erythrocytes of the various groups. Landsteiner and Witt (94) had previously described similar reactions. The specificities or variations disclosed in this way were independent of typical isoagglutination. In Kettel's studies the titer was lowest with homologous erythrocytes, and highest with other erythrocytes of the same blood group. The age of the erythrocytes was found to be important, viz., the sensitivity to cold agglutination diminished on standing at 0°C. when the cells were exposed to saline solution. When in contact with serum, the sensitivity was preserved. All human erythrocytes can be agglutinated in the cold by cold hemagglutinating sera, i.e., all possess receptors for this antibody. The receptors were found in red cells from a 3 month fetus and were well developed at birth. On the other hand, sera of newborns rarely contained cold hemagglutinins.

Nin (141) found receptors for cold hemagglutinins in the lipoids of the stroma of erythrocytes and in the tissue cells of liver and kidney. Nakamura (133) found that lipoids in raw milk cause increases in the titer of cold hemagglutinins when injected into normal rabbits. He stated that the entire reticulo-endothelial system plays an important part in the production of these antibodies. Rosenthal and Corten (153), reviewing the previously held views on the mechanism by which cold hemagglutinins are formed, stated that cold hemagglutination is found only in diseases in which blood destruction is important. Isoimmunization with lipoids of the patient's red cells combined with altered native protein was presented as the mechanism and antigen responsible for the occurrence of cold hemagglutinins. Koepplin (86, 87) described an agglutinogen in the erythrocytes different from all known agglutinogens and peculiar to the erythrocytes of patients with cold hemagglutinins. This was shown by immunization of rabbits with the erythrocytes in question. After appropriate absorption the immune serum agglutinated only the erythrocytes from these patients. The Rh factor was not taken into consideration in these tests.

The following table shows the titer of a single cold hemagglutinating serum (blood group O) using various human red blood cells.

BLOOD GROUP	NUMBER TESTED	TITER 4°C.—½ HOUR				
		1/200	1/400	1/800	1/1600	1/3200
O.....	25	6	3	7	5	4
A1.....	24	7	6	6	1	4
A2.....	2	1			1	
B.....	4		2		1	1
A1B.....	1	1				
A2B.....	1	1				

Heredity

A systematic investigation of the heredity of cold hemagglutinins has not been made. The occurrence of cold hemagglutinins in most, if not all, of the general population would render such a study difficult. The observations which have been recorded in the literature are scanty.

In a case which we studied (169) we had the opportunity to examine 4 children with the following results: one, negative; two, positive 1/10 dilution; one, positive 1/40 dilution.

Duration of the Phenomenon in Disease States

Reference to the tabulation of all the reported cases of cold hemagglutination in humans will reveal:

a. Cold hemagglutination transient:
   1) In acute infectious diseases it has usually been reported as disappearing in convalescence
   2) In trypanosomiasis it disappears in recovery
   3) Acute hemolytic anemia
   4) Some cases of acquired chronic hemolytic anemia
b. Cold hemagglutination permanent:
   1) Cirrhosis of the liver
   2) Raynaud's syndrome or acrogangrene
   3) Some cases of acquired chronic hemolytic anemia

Cold Hemagglutinins in Body Fluids

We were unable to detect cold hemagglutinins in the spinal fluid in one case in which the serum titer was 1/32,000. Absence of hemagglutinins from the spinal fluid was noted by Riebeling (142). Both Milders (119) and Li Chen-Pien (104) found cold hemagglutinins in ascitic fluid in patients with cirrhosis of the liver. Landsteiner (92) found the cold hemagglutinin in artificially produced peritoneal exudates in rabbits. Montanari (128) found cold agglutinins in 3 out of 18 spinal fluids, 2 out of 11 blister fluids, 4 out of 8 transudates or exudates, but not in any gastric, duodenal, or cholecystic fluid.

E. Debenedetti (35) has described the agglutination of spermatozoa by cold hemagglutinins; Benhamou and Nouehy (10) mentioned agglutination of blood platelets; Clough and Richter (26) could not demonstrate agglutination of epithelial cells from the urinary tract obtained by centrifugation of the urine.

Is There a Difference Between Cold Hemagglutinins in Health and Disease?

An attempt has been made by Rosenthal and Corten (153) to differentiate the cold hemagglutinins that occur in normal sera from those that may be present in pathologic sera. They believed that "pathologic cold hemagglutination is not only an increase in the normal but is something different and apart." This question may be discussed under two headings, namely the titers and thermal amplitudes (see below) of the cold hemagglutinins.

Titer. In the study by Kettel, previously mentioned, the highest titer of cold hemagglutinins was 1/1024. Most serum titers were below 1/128 and in 86 per

cent of his cases the titer was 1/16 or lower. Study of the table of the reported cases of cold hemagglutination in disease reveals that in many the titer was not determined; in others, despite the determination of titer, the conditions of the test were not clearly defined, or, if they were defined, they were not standardized. It will be observed, nevertheless, that titers over 1/500 have occurred with some frequency. As mentioned previously, Kettel's series cannot be considered as normals; it seems clear that high titer cold hemagglutinins occur almost exclusively in pathologic conditions. Further study of cold hemagglutinin titers in normals is required. Few authors took the precautions of Kettel to allow blood to clot at 37°C. to prevent absorption of cold hemagglutinins in the clot. Similarly test cell suspensions were not of optimum character. The titers recorded in disease may have been higher if such precautions were observed. In a series of 20 normal bloods whose sera were separated from the clots at room temperature and in which cold hemagglutinins were tested for at 4°C. for one and one-half hours against homologous erythrocytes, we were not able to detect hemagglutinins in 10 and in only one was the titer as high as 1/4.

Thermal Amplitude. The term 'thermal amplitude' was introduced by Bialosuknia and Hirszfeld (12) in 1924 as the temperature range in which the activity of an antibody may be demonstrated. The thermal amplitude of ordinary isoagglutination is from 0°C. to approximately 45–50°C., i.e., agglutination is observed within these limits. The thermal amplitude of the agglutinins in the Hübener-Thomsen phenomenon is intermediate between isoagglutinins and cold hemagglutinins (49). The cold hemagglutinins of most normal sera have a thermal amplitude of 0° to over 10°C. (82). However, normal cold hemagglutinins active at room temperature have been described. 3 per cent of normal sera had thermal amplitudes over 20°C. in Kettel's series. Hirszfeld (69) stated that "the thermal amplitude is not only a question of the titer but of the affinity of the normal antibodies. The agglutinins with great affinity are less dependent on temperature conditions and thus possess a broad thermal amplitude." Amzel and Hirszfeld (3) stated that a broad thermal amplitude of cold hemagglutinins may be found in normal individuals. In another place Hirszfeld (69) stated that a large thermal amplitude is a constitutional characteristic which by itself has no pathological significance. Thomsen (176) has agreed with Hirszfeld on this point. The thermal amplitude of the irregular cold hemagglutinins may be broad and the reactions may exceptionally be observed at 37°C. (89). Clough and Richter (26) pointed out that the upper temperature at which action of a cold agglutinin acts depends upon the mode of testing. In their case, by placing cells and serum at various temperatures, they found an absence of agglutination above 22°C.; i.e., if the temperature were gradually lowered from 37°C., agglutination was first observed at 22°C. However, agglutination brought about at low temperatures persisted to 27°C. There is, therefore, a different ascending and descending threshold of agglutination. There is some agreement, therefore, that the thermal amplitude is not dependent upon any particular measurable value of the serum but is a constitutional peculiarity of the individual.

On the other hand, Mino (121) has stated that the thermal amplitude is

directly dependent upon the titer of cold hemagglutinins. Kettel (82) has carefully investigated the relationship between titer and thermal amplitude and has come to the following conclusion: "The thermal amplitude depends in most, but not in all cases, on the titer. There is an almost proportional relationship, as a rule, between titer and amplitude." Among the 19 of 600 sera which he observed with a thermal amplitude from 0 to over 20°C. but less than 25°C., 4 had low titers at 0° and in the remainder the titers were high (between 1/64 and 1/256—with one at 1/1024). Friedenreich (48) in his monograph stated that the thermal amplitude of the Hübener-Thomsen agglutinin depends directly on the agglutinin content of the serum. At 37°C. this agglutination occurs only with the stronger sera.

From these discussions and other considerations the following conclusions may be drawn:

1. Low titers of cold hemagglutinins occur in normal and pathological sera. Studies have not been carried out to differentiate (if indeed they can be differentiated) these antibodies.

2. Cold hemagglutinin titers at 0 or 4°C. of over 1/500 are rare and may never occur in normal sera. Titers over this level may be considered as definitely abnormal.

3. Though exceptions occur, there is a general direct relationship and proportion between titer and thermal amplitude. The broadest amplitudes occur in pathologic sera because the titers in such sera are usually the highest.

4. Except for the general tendency to higher titers and broader thermal amplitudes of the cold hemagglutinin in pathologic sera, there are no demonstrable differences between such cold hemagglutinins and those in normal sera.

5. There are no systematic studies on the constancy of cold hemagglutinin titers in normal individuals. It is possible that rising or falling titers in disease may be of as much significance as the absolute height of the titer.

Blood Transfusion

There are numerous instances in which blood transfusions were performed without difficulty or reaction in patients with cold agglutinins. This follows from the knowledge that above 30°C. these antibodies (in almost all cases) have no demonstrable activity. There is an occasional report of a transfusion reaction; however, hemolysis or hemoglobinuria have not been described. It seems advisable when transfusing such patients to use blood at or above room temperature and close to body temperature. Warming of the blood by a heat source may bring about hemolysis. The use of hot water bags or other contrivances about the bottle or tubing (in indirect transfusions) does not appear necessary. A biological test has been proposed to insure the fact that the cold hemagglutinin is not masking another antibody which might be capable of causing hemolysis (195). We did not take such a precaution in 6 cases, one with a titer of cold agglutinins of 1/16, one with a titer of 1/128, one with 1/256, and three with 1/1024, in which multiple transfusions were performed without reaction.

In several of the reported cases, cold hemagglutination was observed only after

one or several blood transfusions. In experiments on rabbits Rous and Robertson (151) have shown that occasionally transfusions of blood or repeated bleedings may be followed by the appearance of cold hemagglutinins. Mino (124) did not find a rise in the titer of cold hemagglutinins in humans as a result of blood transfusions.

There have been several reports of the occurrence of irregular cold hemagglutinins in patients who have had previous transfusions. In some of these, the agglutinins were active at 37°C., and may have been responsible for transfusion reactions. Belk (9) has summarized the evidence concerning the role of cold hemagglutinins in transfusion reactions. He concluded that cold hemagglutinins are often the cause of such reactions. It must be pointed out, however, that in none of his cases was evidence of hemolysis, i.e. jaundice, hemoglobinuria, or fall in hemoglobin, observed. His reasoning, that febrile reactions after transfusions are due to cold hemagglutinins because no other cause can be found, is not sound. The great majority of writers on this subject, the individual case reports, and our own experiences indicate that hemolytic reactions do not occur.

As mentioned previously, if the agglutinin has a broad thermal amplitude so that the agglutination and hemolysis can be demonstrated in vitro at temperatures over 35°C., the possibility of a reaction is greater. Apparently even in such cases hemolysis is not common. Under such circumstances the transfusion should be given slowly and the temperature of the infused blood should be close to 37°C.

Laboratory Aspects of Cold Hemagglutination

Technique of cold hemagglutination test

A standardization of the method for testing for cold hemagglutinins would greatly simplify the interpretation of future studies. The following technique, after Kettel (82), has been found dependable and the results reproducible.

1. Venous blood drawn in a dry syringe is placed into a dry sterile tube and into a tube containing oxalate crystals or 3.8 per cent sodium citrate solution. Both tubes are incubated at 37°C., preferably in the water bath, within a few minutes of the venepuncture.

2. After about a half hour the erythrocytes in the tube containing anticoagulant are washed by centrifugation at room temperature or at 37°C., if possible. The supernatant plasma is replaced by at least 3 volumes of isotonic sodium chloride solution. After mixing, this tube is replaced in the water bath for 15 minutes and then recentrifuged. The cells should be washed by this method at least 3 times. After the final washing these cells are not auto-agglutinable at 4°C. If agglutination occurs, repeated washing (as many as 6 washings may be necessary) must be carried out to remove all traces of plasma and cold hemagglutinin. The washed erythrocytes are finally diluted to 1–2 per cent suspension with isotonic sodium chloride solution.

3. As soon as adequate retraction of the clot of the blood in the dry tube has taken place, the serum is carefully removed. An attempt should be made to

remove as few red cells as possible. Centrifugation of the serum at room temperature or at 37°C., if possible, will remove all the erythrocytes.

4. Several hours after the venepuncture, the test for cold hemagglutination is performed. Serological tubes (10 x 75 cm.) are used for this purpose. 0.4 cc. of progressive dilutions of the serum from $1/10^{15}$ to 1/2560 or higher are pipetted into the tubes. Isotonic saline solution is pipetted into the last tube. 0.1 cc. of the red cell suspension is then placed in each tube. After shaking, all the tubes are placed in melting ice (4°C.) and this temperature is maintained for 8 to 15 hours, i.e., overnight. The rack and tubes should be shaken several times during this period.

5. The temperature of 4°C. should be maintained during the process of reading the result. The preliminary reading is by gross examination, the recording symbols being 4 plus to plus-minus to negative. Each tube is inspected after gentle tapping, the hemagglutination recorded, and the tube replaced in the cold rack.

6. The end point is determined by examining the last tube in which clumping was observed macroscopically and the tubes following under the lower power of the microscope. These readings are performed at room temperature and must be carried out rapidly. The end point of agglutination is usually sharp and determined easily.

7. If thermal amplitude tests (see above) are performed, at least 6 temperatures from 4° to 37°C. or rarely higher should be used. The tests are set up as described above. In most cases 4, 10, 15, 22, 30, and 37°C. will be found satisfactory.

8. If the titer of the cold hemagglutinin is being followed during the course of disease, it is important to duplicate the technique of each test so that comparable readings are obtained. The density of the erythrocyte suspension should not vary.

9. If blood drawn on one day is to be tested the subsequent day, the serum must be freed of red cells before storage in the refrigerator. It is advisable to use erythrocytes less than one day old. If erythrocytes must be stored overnight before use, they should be stored in the refrigerator in whole oxalated or citrated blood, rather than in isotonic saline solution. Before using, they must be washed carefully as described above.

AGGLUTININ ABSORPTION[16]

Following Landsteiner and others, an experiment of agglutinin absorption was performed to determine whether the cold hemagglutinin is absorbable, how much absorption is required to remove it from the serum, and whether the agglutinated cells release the agglutinin under proper conditions. To investigate these points 6 cc. of the patient's serum were mixed with one cc. of washed packed homologous erythrocytes and kept at 4°C. for 6 hours. At this time the serum was removed, one cc. saved for cold hemagglutinin titration and the re-

[15] At this dilution rouleaux formation does not occur. If lower dilutions are used, rouleaux formation must be excluded by microscopic examination.

[16] Performed at Harlem Hospital.

mainder exposed to one cc. of fresh washed homologous erythrocytes. After 6 hours in the cold the serum was again removed, 1 cc. saved and the remainder mixed with fresh erythrocytes. This procedure of repeated agglutination of fresh erythrocytes with the same sample (decreasing amounts) of the patient's serum was performed 6 times. The agglutinated erythrocytes from each agglutination were saved at 4°C. At the conclusion of the experiment the cold hemagglutinin titer of each of the samples (representing progressive absorptions of a single serum) was determined.

The original serum titer was over 1/2048. The serum titer after each of the first 3 absorptions was over 1/2048. The sera of the fourth and fifth absorptions were lost. The titer after the sixth absorption was 1/512. These results emphasize the difficulty which exists, when the titer is high, of exhausting the serum of its agglutinins. The experiment shows that the agglutinin is absorbable.

The 6 samples of cold agglutinated red blood cells were washed 3 times with ice cold 0.85 per cent sodium chloride solution. Each of these washings was saved and tested for cold hemagglutinins. The first washing from each had a cold hemagglutinin titer of 1/8 to 1/16. The subsequent washings had lower titers and in several the third washing did not contain any demonstrable cold hemagglutinins. These results show that the agglutinins removed from the original serum by the erythrocytes cannot be washed away by cold saline solution.

The washed cold agglutinated cells from the last experiment were then placed in the water bath at 37°C. for one hour. About 1.5 cc. of 0.85 per cent sodium chloride was added to each tube. The massive agglutination broke up quickly and free dispersion of the cells resulted. After centrifugation at room temperature, the supernatant clear saline solution was removed from each tube and tested for cold hemagglutinins. In each of the 6 instances the titer of cold hemagglutinins was over 1/2048. This experiment demonstrates that the cold hemagglutinins can be released by warming agglutinated cells to 37°C. This property of cold hemagglutination will be utilized in a subsequent experiment in which antibody nitrogen determinations are reported. The following figure is a summary of these findings of cold hemagglutinin absorption.

THE HEMOLYTIC MECHANISM IN COLD HEMAGGLUTINATION.

Remarks from the Literature

In the great majority of cases of cold hemagglutination that have been reported, including 4 personally observed cases, hemolysis in vitro could not be demonstrated. In few, if any, were complete studies on this fundamental point carried out. Bonnard (16) in a review of cold hemagglution stated that the absence of auto and isolysis seemed to be constant.

As has been pointed out previously, in a few instances of cold hemagglutination experimental exposure of the patient to cold (155, 160, 169) resulted in intra vitam hemolysis. In some of the other cases of hemoglobinuria with cold hemagglutination a close clinical relationship between the cold, the hemagglutinin, and intra vitam hemolysis could be readily established.

There is a sharp discrepancy, therefore, in many cases in that the production

of hemolysis in vivo could not be paralleled by in vitro tests demonstrating the same phenomenon. Reference to the few instances in which hemolysis was shown in vitro will illustrate the variability of the conditions under which it occurred. We are therefore confronted with a situation in which strong hemolysis can be readily demonstrated in vivo while only hemagglutination can be produced regularly in vitro. There can be no question that a hemolytic action of the agglutinin occurs. The mechanism of this action is not clear (see below).

The most complete investigation of this question was carried out by Salén (160).[17] He found that, if the supernatant plasma of a cold mixture of erythrocytes and plasma was removed and replaced with cold saline solution, hemolysis resulted. The plasma protected the cells against hemolysis; upon its removal and replacement with saline, lysis of the agglutinated cells resulted. However the agglutinin suspended in saline solution did not cause hemolysis. D'Antona (33) stated in a case with a cold hemagglutinin titer of 1/1280 that hemolysis resulted if serum diluted 1/5 or 1/10 was mixed with homologous erythrocytes. Brulé et al. (20) stated that when agglutinated erythrocytes were washed with 0.7 per cent sodium chloride solution hemolysis began after the third washing. Stieffel (case 4) (172) stated that deplasmatized red cells were hemolyzed even in hypertonic saline solutions. Wyschegorodzewa (200) demonstrated auto and isohemolysins at 37° in a case with cold hemagglutinins. Complement was necessary for the lysis. Reisner and Kalkstein (147) in a case of cold hemagglutination with an unusually high thermal amplitude stated that they believed that an autohemolysin was present even though they could not demonstrate it They stated that the intense hemagglutination prevented the lysis in vitro. Neuda (139) stated that an autolysin always occurs with the autoagglutinin but cannot be demonstrated easily because of the presence of inhibitors. He suggested that dilutions of serum in the range of 1/16 to 1/64 be mixed with red cells and placed in the cold for one hour. After 24 hours at 37°C. hemolysis results.

In an attempt to elucidate the mechanism of hemolysis, intensive study of a single case (previously reported) was carried out.

Cold Hemolysis in Vitro[18]

The following summary of hemolysis in vitro in 2 cases with cold hemagglutinin titers of between 1/20,000 and 1/30,000 at 4°C. is taken from material published in detail in another place (206).

The hemolysis demonstrated by these experiments in the cold is fundamentally different from that in the Donath-Landsteiner test. The latter test is one in which hemolysis occurs only after warming a chilled specimen of blood. In that test, the hemolysis is dependent upon complement fixation in the cold and lysis of the red blood cells sensitized by the amboceptor. The experiments summarized above differ in that the hemolysis occurs only in the cold; hemolysis is strictly dependent upon the intensity of agglutination (for slight dilution of the

[17] The details of this careful study are analyzed in another publication (206).

[18] Performed at The Mount Sinai Hospital.

TABLE III

Cold Hemolysis with Cold Hemagglutination

Refer to explanatory notes at end of the table. All tubes kept at 4°C. for 30 min.

SPECIMEN	TRAUMA AT INTERVALS OF	AGGLUTINATION	HEMOLYSIS
	min.		
Whole blood 0.5 cc.	5	marked	4+
Whole blood 0.5 cc.	15	marked	2+
Whole blood 0.5 cc.	no trauma	marked	0
Fresh plasma 0.3 cc. + 80 per cent RBC 0.2 cc.	5	marked	4+
Fresh plasma 0.3 cc. + 60 per cent RBC 0.2 cc.	5	marked	2+
Fresh plasma 0.3 cc. + 40 per cent RBC 0.2 cc.	5	marked	+
Fresh plasma 0.3 cc. + 20 per cent RBC 0.2 cc.	5	marked	0
Inactivated plasma 0.3 cc. + 80 per cent RBC 0.2 cc.	5	marked	4+
0.85 per cent NaCl 0.3 cc. + 80 per cent RBC 0.2 cc.	2	slight	0
Fresh plasma 1/10 dil. 0.3 cc. + 80 per cent RBC 0.2 cc.	5	marked	0
Fresh plasma 0.3 cc. + 80 per cent normal RBC 0.2 cc.	5	marked	4+
Normal whole blood 0.5 cc.	2	none	0
The following experiment carried out at 37°C.			
Whole blood 0.5 cc.	2	none	0
The following experiment started at 4°C. for 15 min. and completed after 1 hr. at 37°C.			
Whole blood 0.5 cc.	none at 4°C. gentle at 37°C.	none	0

1. These experiments were set up at 37°C. in 10 x 75 mm. serological tubes and then transferred to the indicated temperature.

2. The "trauma" refers to a standard shaking at the stated intervals as follows: the tube was held upright at the top and was tapped gently with the finger at the bottom 20 times in rapid succession. It was then replaced in the rack.

3. The results were read with minimal agitation. Agglutination was observed by tilting the tube and watching the stream of erythrocytes flow up and down the tube. If sufficient sedimentation had occurred, hemolysis was read directly; if not, centrifugation at room temperature at 600 R.P.M. for 3 minutes preceded the reading.

4. Whole blood—oxalated blood of the patient with cold hemagglutinins tested within one hour of venepuncture and kept at 37°C. until use.

5. Fresh plasma—oxalated of patient with cold hemagglutinins.

6. Inactivated plasma—above heated to 56°C. for 40 minutes.

7. Diluted plasma—above diluted with either 0.85 per cent sodium chloride solution or normal plasma of the same blood group.

8. Duplicate experiments with serum separated from blood clotted at 37°C. yielded the same results as plasma.

9. RBC—unwashed packed homologous red blood cells except where normal red blood cells were used. These were from healthy donors of the same blood group as the patient tested.

10. The symbols 4 + to 0 under 'Hemolysis' refer to the degree of hemolysis, the former indicating intense hemolysis and the latter no hemolysis.

serum or plasma abolishes the phenomenon); the degree of hemolysis diminishes as the concentration of red blood cells is diminished (even though the intensity of agglutination is greater); hemolysis occurs only when mechanical trauma is added to agglutination in the cold. Other features of this reaction will be discussed more fully in another publication.

To date we have had the opportunity of testing the effect of trauma upon cold agglutinated erythrocytes in only 8 cases. The 2 in which the phenomenon was demonstrated were similar in that the cold hemagglutinin titers were identical (vida supra) and unusually high. It is of great interest, however, that these cases had no clinical similarities. One case[19] was a patient with severe acute hemolytic anemia following a virus (?) pneumonia (recovery from both conditions occurred promptly), while the other was a patient, mentioned previously, with symmetrical gangrene of the tips of his fingers and toes. He did not have a hemolytic anemia whereas the previous patient did not have gangrene or a Raynaud syndrome. The third patient, in whom the hemolytic mechanism was not demonstrable, had recovered from a splenectomy for hemolytic anemia

TABLE IV

	Per cent NaCl												
	0.85	0.72	0.64	0.56	0.52	0.48	0.44	0.42	0.40	0.38	0.36	0.32	0.28
Control	0	0	0	0	0	tr	+	2+	3+	3+	3+	4+	4+
Warm	0	0	0	0	0	0	+	2+	2+	3+	3+	3+	3+
Cold	tr	tr	tr	tr	tr	±	+	2+	3+	3+	3+	3+	4+

0, no hemolysis; tr, trace of hemolysis; ±, slight hemolysis; +, 2+, 3+, increasing degrees of hemolysis; 4+, complete hemolysis.

The trace of hemolysis in the cold blood above 0.48% sodium chloride solution was due to the occurrence of intra vitam hemoglobinemia. The fragility of the 3 samples was substantially the same.

associated with cold hemagglutinins. However, the titer of the hemagglutinins in this case was lower, 1/1028. The fourth and fifth cases had a titer of cold hemagglutinins of 1/80. The sixth case was one of hemolytic crisis in sickle cell anemia with a cold hemagglutinin titer of 1/1028. The other cases followed atypical pneumonia and had titers of 1/256.

Erythrocyte Fragility Test[20]

Hypotonic Saline Fragility. Duplicate tubes were set up containing 2 cc. of dilutions of sodium chloride solution. 0.1 cc. of the patient's serum was added to each tube of one set while a similar amount of group O normal serum was added to each tube of the second set. The tubes were placed in the refrigerator for 2 hours. 0.1 cc. of a 20 per cent suspension of the patient's cold washed cells

[19] Mount Sinai Hospital #503906.

[20] In this and in the subsequent experiments with saponin, erythrocyte volume and complement absorption during hemagglutination in the cold, the titer of cold hemagglutinins was 1/30,000 (169). These studies were carried out at Harlem Hospital.

(group O) were added to each tube. The tubes were kept at 10°C. for one hour. The degree of hemolysis in each tube was read after centrifugation. Hemolysis in each set was identical, starting at 0.46 per cent sodium chloride and being completed at 0.30 per cent sodium chloride.

In another experiment 2 cc. of a 20 per cent suspension of the patient's red cells was mixed with 1 cc. of the patient's serum in one tube and with 1 cc. of normal group O serum in another. The tubes were placed at 8°C. for 3 days. After this time the tubes were placed in the water bath at 37°C. for one hour. The hemagglutination of the erythrocytes exposed to the patient's serum disappeared. The tubes were then placed at 4°C. for one hour. The previously agglutinated cells were reclumped. The tubes were then placed at 37°C. for one hour. After this time 0.1 cc. of erythrocyte mixture from each tube was added to progressive dilutions of sodium chloride solution at 37°C. so that duplicate sets were formed. Hemolysis in each started at 0.50 per cent and was complete at 0.36 per cent sodium chloride.

Three samples of the patient's venous blood were obtained. The first sample (control) was withdrawn at the beginning of the test. The right arm of the patient was then immersed in ice water (as in the previously described experiment on hemoglobinemia) for 20 minutes. Upon removal of this arm from the cold, blood was withdrawn from the right (cold) and left (warm) arms. Coagulation was prevented by use of 3.8 per cent sodium citrate. 0.1 cc. of blood of each of the 3 samples were added to progressive dilutions of sodium chloride solution.

Saponin Fragility

The following experiment was performed to observe the effect of saponin upon agglutinated and normal erythrocytes.

Tube 1. 2 cc. 10 per cent patient's erythrocytes plus 2 cc. 1/10 dilution patient's serum.

Tube 2. 2 cc. 10 per cent patient's erythrocytes plus 2 cc. 1/10 dilution group O normal serum.

These tubes were placed at 4°C. for one hour, then warmed to 37°C. for one-half hour, then cooled to 4°C. for one hour, and finally warmed to 37°C. for one hour. Upon each exposure to low temperatures marked hemagglutination occurred in the tube containing the patient's serum. 0.2 cc. from each was added to 2 cc. of progressively diminishing concentrations of saponin in 0.85 per cent saline solution from 0.1 to 0.002 per cent saponin. In each, hemolysis started in approximately 0.007 per cent saponin and was complete in 0.025 per cent.

In other experiments the fragility of agglutinated and normal erythrocytes was tested by shaking with glass beads for one hour in a mechanical shaker and by the use of complement and an anti-human erythrocyte serum. The liability of the erythrocytes to be hemolyzed by either of these tests was not affected by previous agglutination.

Erythrocyte Volume

It is well known that erythrocytes assume a spherical form before hemolyzing in hypotonic salt solution and probably in other agents. An experiment was

performed to determine whether erythrocytes exposed to the cold agglutinating serum increased in size. This would provide indirect evidence bearing on the question of whether agglutination injures the cells. The most convenient means of measuring this is to determine the packed red cell volume of red cells suspended in the serum at various temperatures. The red blood cell volume of a particular sample of blood at room temperature was 21 per cent whereas at 8°C. it was 23.2 per cent. The agglutinated cells occupied a greater volume than the normal cells. The interpretation of this fact is open to the criticism of inadequate packing of the large masses of agglutinated cells at the lower temperature. The previously presented evidence all points to the fact that agglutination does not affect erythrocyte fragility and thus, indirectly, cell volume.

Complement Absorption During Hemagglutination[21]

In a previous section of this paper it was suggested that hemolysis by antibodies occurs only when complement participates in the reaction. It was stated that the cold hemagglutinin does not take up complement when it combines with erythrocytes and that it is for this reason that it does not produce hemolysis. Neuda (140) stated that complement activity of an absorbed serum may be greater than that of an unabsorbed serum. The following experiment is designed to illustrate this point.

Four tubes were prepared as follows:

1. 10 per cent group O washed erythrocytes, guinea pig serum (complement), patient's serum inactivated, 1/20 dilution.
2. 10 per cent group O washed erythrocytes, guinea pig serum (complement), normal group O serum inactivated, 1/20 dilution.
3. 10 per cent group O washed erythrocytes, guinea pig serum (complement), saline solution.
4. 10 per cent group O washed erythrocytes, saline solution, patient's serum inactivated, 1/20 dilution.

The tubes were all iced for 20 minutes, centrifuged in the cold, the supernatant solutions removed and accurately titered for complement content using sheep erythrocytes and a rabbit anti-sheep erythrocyte serum. Complement titrations were identical in tubes 1, 2, and 3. Marked hemolysis occurred above a dilution of 1/40, indicating the presence of complement in all supernatants. No hemolysis was produced by the supernatant from tube 4. (It is realized that the possibility exists that human complement may have behaved differently in regard to absorption in the cold during hemagglutination. This was not studied.)

Conclusions

Experiences with other substances indicate that a serological test does not always mirror conditions within the body. Broad conclusions regarding the effect of a substance in in vitro testing cannot be carried over to the animal organism in many instances. The inability to reproduce in vitro (except with high titer cold hemagglutinins) the hemolysis which is so evident in vivo is not peculiar to cold hemagglutinins. Ham and Castle (63) in experiments with concanavalin A (extracted from the jack bean) found strong erythrocyte agglu-

[21] Performed at Harlem Hospital.

tination without hemolysis in vitro. When the material was injected into animals, hemolysis of the red blood cells and hemoglobinuria resulted. In accord with their theory that erythrostasis is an important factor in hemolysis in vivo, they cited other instances in which strong agglutination of erythrocytes with little or no hemolysis in in vitro experiments was paralleled by intense in vivo hemolysis by the same agent. According to Levine (211) anti-Rh serums produce only agglutination in vitro, failing to hemolyse erythrocytes. In vivo they may cause intense hemolytic reactions.

The recent experiences with transfusion reactions due to Rh incompatibility have demonstrated that marked reactions may occur, if an Rh negative recipient who has had transfusions of Rh positive blood (and has thus been immunized), receives another transfusion of Rh positive blood, even if the test tube reaction fails to demonstrate an incompatibility. The fact that anti-Rh agglutinins cannot be demonstrated in such a patient does not mean that marked intravascular hemolysis will not result (196). In such instances in which in vitro tests do not demonstrate incompatibility, a biological test has been proposed (197).

Furthermore, in the case of almost all incompatible blood transfusions, the incompatibility is tested by hemagglutination reactions. In vitro considerable hemagglutination and slight or no hemolysis is observed; in vivo the degree of hemolysis is marked and hemagglutination occupies a very minor position. We were not able to detect intravascular agglutination in the tissues of 6 patients who died after hemolytic transfusion reactions.

The fact that potent agglutination was demonstrable in vitro without hemolysis when the cold hemagglutinin titer was low or moderate, whereas marked hemolysis occurred in vivo, is of great theoretical interest. These mechanisms may be related to hemolysis in general and to hemolytic anemia (29, 30). The detailed evidence that agglutination results in no regularly demonstrable injury to the erythrocytes has been presented. On the basis of the techniques now available for the study of erythrocytes, the conclusion is warranted that some other factor or mechanism besides agglutination operates to cause hemolysis within the animal organism.

The key to one of these factors, which hitherto has received very little consideration in hemolysis, is provided by the experiments in which hemolysis of cold agglutinated erythrocytes was effected by gentle shaking. This indicates that an immuno-mechanical mechanism may at times be of importance. There can be little question but that erythrocytes are exposed to considerable trauma while circulating.

The hemolytic mechanism operative in some instances of hemolytic anemia may not be based entirely upon immunological reactions.

THE EFFECT OF EXTERNAL TEMPERATURE ON THE SEDIMENTATION RATE OF ERYTHROCYTES[22]

Introduction

General Remarks. Fahraeus (45) has shown that of the various factors which affect the speed of settling (sedimentation) of red blood cells in blood.

[22] Performed at The Mount Sinai Hospital.

i.e., the specific gravity of the corpuscles, the specific gravity of the plasma, the viscosity of the plasma, the size of the corpuscles, and the presence of aggregates of corpuscles, the latter is the most important. He has shown a direct relationship between the size of rouleaux aggregates and the sedimentation rate in a variety of conditions in humans and animals. Ham and Curtis (64) stated that "any substance which affects the degree of erythrocyte aggregation will alter the sedimentation rate." In neither of these publications is the question of the effect of cold hemagglutination upon the sedimentation rate discussed.

There are very few observations in the literature concerning the effect of external temperature upon the sedimentation rate. DeCourcy (37) tested the sedimentation at 6°, 18°, and 37°C. and found a small difference in sedimentation between 6° and 18°C. but a great difference between 18° and 37°C. Gordon and Cohn (56) performed sedimentation rate tests at 10°, 23°, and 37°C. They found an acceleration of sedimentation with an increase in temperature and a marked diminution with a decrease. Westergren (186) has tested the rate of sedimentation of blood at temperatures between 14 and 25°C. At slow sedimentation rates, slight increases in sedimentation with increasing temperature were observed over this range. However at rapid rates the increase was proportionally greater. To control this variable in the performance of the sedimentation rate, he proposed 18°C. as the temperature at which all such tests should be performed.

Sedimentation in Reported Cases of Cold Hemagglutination. Jessen and Bing (78) were the only authors who studied the relationship. In a case in which an extremely potent cold hemagglutinin was present, they found a sedimentation of 106 mm. in one hour at 19°C. and 55 mm. in one hour at 40°C. In a case of uremia with a much lower titer of cold hemagglutinins they reported a sedimentation of 114 mm. in one hour at 19°C. and 67 mm. in one hour at 40°C. As a comparison they cited the sedimentation in a case of polyarthritis without cold hemagglutination at 120 mm. in one hour at 19°C. and 140 mm. in one hour at 40°C.

Isolated mention of the sedimentation rate in cases with cold hemagglutination is noted in the following: Stillman (173) found a sedimentation of 24 mm. in 14 minutes at 37°C. and 24 mm. in 21 minutes at 22°C.; Riebeling (149) found a very rapid sedimentation rate (temperature not mentioned); Thiodet and Ribère (175) reported a rate of settling of 114 mm. in one hour (temperature not mentioned); Koepplin (86) found a rate of 112 mm. in one hour (temperature not mentioned); Rosenthal and Corten (153) reported a maximum sedimentation after 10 minutes (temperature not mentioned); Galli and Mussafia (51) found a sedimentation of 38 mm. in one hour and 75 mm. in 2 hours at 37°C. and 110 mm. in one hour and 135 mm. in 2 hours at 5°C. Reisner and Kalkstein (147) reported a sedimentation rate of 80 mm. in 5 minutes. In their first case Benians and Feasby (11) reported a sedimentation of 120 mm. in 30 minutes at winter room temperature and 12 mm. in one hour at 37°C. These references make no pretext to be a complete bibliography of this phase of the subject.

Many of these patients were anemic or had other conditions which might affect the sedimentation of the corpuscles.

Experimental Study

We have studied the effect of external temperature on the sedimentation rate of various bloods, some containing cold hemagglutinins and others from normal and diseased individuals. We shall not attempt at present to elucidate the intricacies of this subject since they are beyond the scope of this paper.

In all the following examples venous blood was mixed with 1/10 volume of 3.8 per cent sodium citrate solution. The sedimentation of the corpuscles was measured in mm. at 5 or 10 minute intervals for 30 to 60 minutes. The tests were performed at the temperatures indicated in tubes 4 x 100 mm. (In all tests there was a delay of several minutes from the time the sedimentation tube was filled until the required temperature was attained.)

(1) Normal Blood. Hematocrit 40 per cent.

Temperatures	10 min	20 min.	30 min.	40 min.	50 min.	60 min.
°C.						
4	0	0	0.25	0.25	0.5	0.5
9	0	0	0.25	0.5	1.0	1.25
16	0	0.25	0.5	1.0	1.25	1.75
22	0	0.5	1.0	1.5	2.0	2.5
37	0.5	1.0	1.75	3.5	4.5	6.5

(2) Blood with Increased Globulin Content—Multiple Myeloma. Hematocrit 33 per cent.

Temperatures	5 min.	10 min.	15 min.	20 min.	25 min.	30 min.
°C.						
4	1	1.5	3	5	10	15
14	1	4	20	48	52	56
22	4	21	45	55	56	58
37	9	44	57	59.5	60	60

(3) Blood with Increased Fibrinogen Content—Diabetes Mellitus, Cellulitis of Foot. Hematocrit 37 per cent.

Temperatures	10 min.	20 min.	30 min.	40 min.	50 min.	60 min.
°C.						
6	3	5	6	10	26.5	33
14	3	19	37	46	51.5	52
22	14.5	31	49.5	53	55	55
37	26.5	50	54	55.5	57	57

(4) Normal Blood with Isoagglutination. a. *Normal anti-A serum inactivated and group A cells.* Hematocrit 38 per cent.

Temperatures	10 min.	20 min.	30 min.	40 min.	50 min.	60 min.
°C.						
4	0	0	0.25	0.5	0.75	1.0
14	0	0.5	0.25	1.0	1.25	1.5
22	0.5	0.5	0.75	1.5	1.75	2.0
37	0.5	1.0	1.0	2.0	3.0	4.0

b. *Normal anti-A serum inactivated and group B cells.* Hematocrit 41 per cent. The figures for this experiment are given for comparison with those above.

Temperature	10 min.	20 min.	30 min.	40 min.	50 min.	60 min.
°C.						
4	0	0	0	0	0	0
14	0	0.25	0.25	0.5	0.75	0.75
22	0.25	0.25	0.5	0.75	1.0	1.5
37	0.5	0.5	0.75	1.25	1.5	2.0

(5) Blood with Cold Hemagglutinins. a. *Cold hemagglutinin titer 1/3000 at 4°C.* Gangrene of the fingers and toes. Hematocrit 42 per cent. (Harlem Hospital case.)

Temperature	10 min.	20 min.	55 min.	65 min.
°C.				
4	1.0	1.5	9.0	11.0
8	1.0	5.5	19.0	22.0
16	2.0	10.0	28.0	30.0 ✓
22	0.5	2.0	9.0	11.0
37	3.0	11.0	40.0	45.0

At another time the sedimentations of defibrinated blood in this case were as follows (60 minutes):

at 4°C..... 5.0 mm.
at 8°C..... 20.0 mm.
at 12°C..... 20.0 mm.
at 20°C..... 3.0 mm.
at 37°C..... 21.0 mm.

On retesting this blood the following sedimentations were observed at the end of 64 minutes:

at 4°C..... 8.5 mm.
at 7°C..... 13.0 mm.
at 17°C..... 29.0 mm.
at 22°C..... 9.0 mm.
at 37°C..... 44.5 mm.

b. *Cold hemagglutinin titer 1/16 at 4°C.* Diffuse lymphosarcomatosis. Hematocrit 39 per cent. (Mount Sinai Hospital #497192.)

TEMPERATURE	10 MIN.	20 MIN.	30 MIN.	40 MIN.	50 MIN.	60 MIN.
°C.						
4	0	0.5	0.5	0.5	1.5	2.5
14	0	0.5	1.0	1.5	3.5	5.5
22	0.5	1.0	3.0	4.0	6.0	7.5
37	1.0	2.5	5.0	7.5	10.0	12.0

c. *Cold hemagglutinin titer 1/1280 at 4°C.* Status post splenectomy for acquired hemolytic anemia. Pregnancy 6 months gestation. Hematocrit 36 per cent. (Mount Sinai Hospital #416010.)

TEMPERATURE	10 MIN.	20 MIN.	30 MIN.	40 MIN.	50 MIN.	60 MIN.
°C.						
4	0.5	1.0	1.5	1.75	2.0	2.5
7	1.0	1.5	2.0	2.0	2.5	3.0
17	1.5	2.0	2.75	3.0	3.0	3.5
22	0.5	1.0	1.5	2.0	3.5	4.5
37	1.0	6.0	26.0	36.0	40.0	44.0

Summary and Conclusions

The summary and conclusions drawn from this study of the effect of external temperature on the sedimentation rate of blood are as follows:

1. Extreme cold (4°C.) had a marked retarding effect on sedimentation of all the bloods tested. This applied as well to blood with marked rouleaux formation (high globulin and fibrinogen content) and with marked cold hemagglutination. Despite the large size of the red blood cell aggregates in the latter blood, sedimentation was slow.

2. Body temperature (37°C.) had a marked accelerating effect on the sedimentation of all the bloods tested. This was observed in the first 20 minutes of sedimentation in bloods with high globulin or fibrinogen content but was observed to a considerable extent in all.

3. In all the bloods except 2 of the cases of cold hemagglutination there was a progressive increase in the rate of sedimentation from 4 to 37°C.

4. In the 2 bloods with high titers of cold hemagglutinins there was a significant increase in the sedimentation rate at about 16°C. and a slowing (relative in one case, and absolute in the other) at about 22°C. This paradoxical effect is due to the presence of the cold hemagglutinins. In each of these cases the thermal amplitude of the agglutinins extended up to about 25°C. with a considerable fall in titer between 16 and 22°C.

5. The slow sedimentation at temperatures below 14°C. of blood with cold hemagglutinins is not explainable from the present data. At these temperatures the degree of erythrocyte aggregation is marked. A slow rate of sedimentation was also observed in a tube 8 mm. in diameter.

CHEMICAL AND PHYSICAL PROPERTIES OF COLD HEMAGGLUTININS

Introduction

Landsteiner (92) was the first to investigate this question of the chemical constitution of the cold hemagglutinin. He stated that the autoagglutinin of normal rabbit serum is in the globulin fraction of the serum proteins. Clough and Richter (26) added 36 volumes % of saturated ammonium sulfate to a human serum containing cold hemagglutinins and stated that all the antibody was found in the precipitate following the salting-out procedure. They stated that the antibody is in the euglobulin fraction. Mino (120) stated that carbon dioxide saturation of serum did not precipitate the cold hemagglutinins. Koepplin (86, 87) stated that the cold agglutinin is bound to the serum globulin since it was precipitated by saturated ammonium sulfate.

TABLE V

SPECIMEN	TITER COLD HEMAGGLUTININS, 4°C.	TOTAL NITROGEN
		mg./cc.
1st saline washing of agglutinated ghosts	1/40	0.614, 0.722
2nd saline washing of agglutinated ghosts	1/20	0.153, 0.146
3rd saline washing of agglutinated ghosts	1/10	0.059, 0.060
4th saline washing of agglutinated ghosts	1/10	0.035, 0.028
Resuspended antibody	1/2560	1.483, 1.464
Ghost Control Washing		0.004, 0.004
Blank for Reagents		0.027, 0.034

Blank has been deducted from results.

Antibody Nitrogen Studies

Stats, Perlman, Bullowa and Goodkind have reported in another publication (168) the details of a study performed on the serum of a recent case with a titer of cold hemagglutinins which varied from 1/3000 to 1/32,000 at 4°C.

Briefly, erythrocyte ghosts practically free of hemoglobin were prepared by repeated hemolysis of a single sample of serum-free erythrocytes. The light pink ghosts were strongly agglutinated at 4°C. for 12 hours by a cold hemagglutinating serum and were then separated from the serum by centrifugation at 4°C. and successive washings with ice cold 0.85 per cent sodium chloride solution.

The saline washings of the agglutinated ghosts were tested for cold hemagglutinin content and for total nitrogen. The washed cold agglutinated ghosts were then exposed to a temperature of 37°C. for one hour in the presence of a small quantity of normal saline solution. At the end of this time, the ghosts were freely dispersed but were sedimented by centrifugation at room temperature. The clear saline eluate (resuspended antibody) was tested for hemagglutinin content and for total nitrogen. Control specimens of saline solution and washings from unagglutinated ghosts were obtained (ghost control washing).

In Table V there is a summary of this work taken from the previous publication (168).

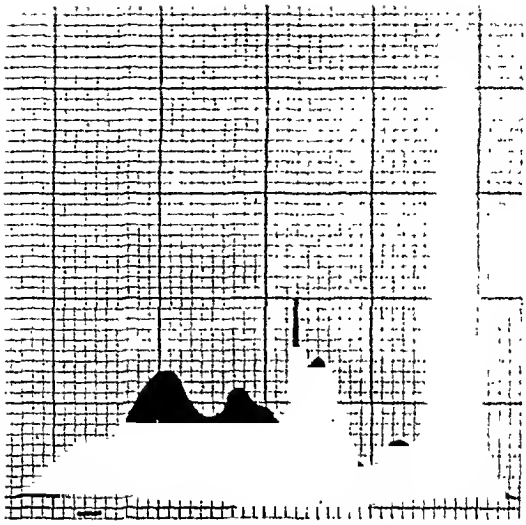

FIG. 4. DESCENDING ELECTROPHORETIC PATTERN OF UNABSORBED SERUM

Reproduced by courtesy of the Editors of the Proceedings of the Society for Experimental Biology and Medicine (168).

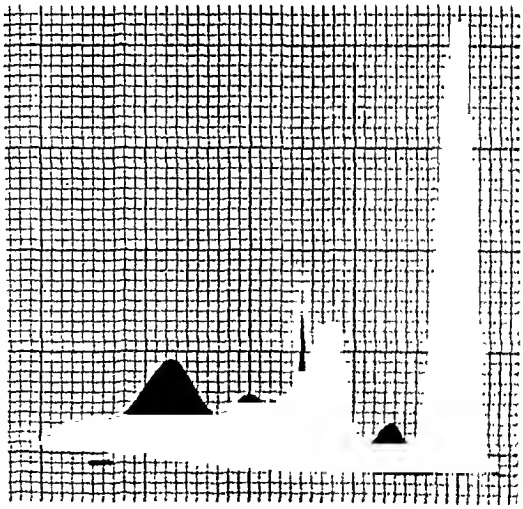

FIG. 5. DESCENDING ELECTROPHORETIC PATTERN OF ABSORBED SERUM

Reproduced by courtesy of the Editors of the Proceedings of the Society for Experimental Biology and Medicine (168). There is a 16 per cent decrease in the γ globulin, the other components retaining an unchanged relationship to the albumin concentration.

The total nitrogen content of the resuspended antibody probably represents only antibody nitrogen. However, there may have been an unknown quantity of non-antibody protein non-specifically absorbed by the ghosts during cold hemagglutination. There is also the possibility that serum protein was enmeshed in the agglutinated cell mosaic and was released when the agglutination broke up at 37°C.

Electrophoresis

This determination was previously reported by one of us with the preceding material on antibody nitrogen (168).

The electrophoretic studies were conducted by preparing 2 sera, one the serum of the patient, and the other the serum of the patient absorbed 6 times in the cold with erythrocyte ghosts. The titer of cold hemagglutinins in the original unabsorbed serum was 1/2560 at 4°C. whereas the absorbed serum had a titer of 1/320. Each of these sera was then diluted with buffer to reduce the total protein content to between 1 and 1.5 per cent. They were then subjected to prolonged dialysis against the buffer in the refrigerator. In this experiment a diethylbarbituric acid sodium hydroxide buffer of pH 8.63 and ionic strength of 0.10 was used.

Figures 4 and 5 represent the electrophoretic patterns of the unabsorbed and absorbed serum. By planimetric measurements, using the technique of Longsworth, Shedlovsky, and MacInnes (106), it can be shown that there is a significant difference between the gamma globulin portions of each curve, indicating absorption of a fraction of this component. The inference is that the cold hemagglutinin has the mobility of gamma globulin (129).

Summary and Conclusions

1. The serological characteristics and terminology of cold hemagglutination, autohemagglutination, pseudoagglutination or rouleaux formation, and panagglutination are discussed.

2. A serological reaction in which the following features are observed should be denoted "cold hemagglutination":

   a. Agglutination of homologous or heterologous erythrocytes at low temperatures with complete reversal of the reaction upon increasing the temperature (to 25°C. or less in most cases). Chilling of the resuspended cell-serum mixture again results in agglutination.

   b. Exhaustion of the agglutinins in the serum by absorption with erythrocytes at low temperatures.

   c. Release of the agglutinins from the cold agglutinated erythrocytes by raising the temperature to approximately 37°C.

3. Atypical cold hemagglutinin sera are mentioned.

4. Table I presents in summary all of the reported cases of cold hemagglutination in disease and mentions the titer of agglutinins, clinical diagnosis, and pertinent data in each instance.

5. The recognition of cold hemagglutination during the performance of red blood count or blood grouping determination is described and methods are discussed by which the phenomenon can be dissipated. The differences between

these observations and those in hyperglobulinemia or marked rouleaux forma are discussed.

6. The occurrence of cold hemagglutination in acute infectious diseases is cussed. There is no adequate study of this relationship. The hemagglutin is transient in these cases and usually does not attain a very high titer.

7. The occurrence of cold hemagglutination in hematologic disorders, espec in hemolytic anemia, is discussed. The infrequent association of these co tions is mentioned. The variability of the hematologic findings in such cas pointed out. The phenomenon may not have any real relationship to the un lying anemia in some cases, for the anemia may be cured by splenectomy bu hemagglutinin persists. Some cases of acute hemolytic anemia due to sulf mides showed transient agglutinins.

8. The presence of cold hemagglutination in cases of hemoglobinuria related to exposure to cold is observed. We do not believe that in those ca relationship existed between the agglutinins and the hemoglobinuria.

9. The presence of cold hemagglutination in cases of paroxysmal hemog nuria following exposure to cold (paroxysmal cold hemoglobinuria) is pointed The mechanism of the hemoglobinuria is demonstrated in an experimen which hemoglobinemia occurred after exposure of the forearm to cold. probable in these cases that the hemoglobinuria is due to the cold hemagglut

10. A detailed analysis of the differences and similarities between paroxy cold hemoglobinuria associated with cold hemagglutination and paroxy cold hemoglobinuria as found in syphilitics (Donath-Landsteiner) is presen It is concluded that these syndromes differ in many respects and that should be separated. The cold hemagglutination cases are rare.

11. The frequent occurrence of the phenomenon is trypanosomiasis (hu and animal), spirillosis (animal), and the infrequent occurrence in piroplasm (canine) is summarized. Cold hemagglutination and malaria is a puz problem; the data available are not adequate to pass final judgment. The liminary conclusion is that the phenomenon occurs rarely, if at all, in close ciation with malaria.

12. The relationship of the phenomenon to "hypertrophic" or "infect cirrhosis of the liver is pointed out.

13. The frequency of the Raynaud's syndrome in association with the nomenon is indicated. The mechanism is elucidated by observation of slo and discontinuity of the blood flow in the conjunctival vessels after irriga of the sac with cold saline solution.

14. Three cases of cold hemagglutination in pregnancy are discussed.

15. The relationship between agglutination and bland venous thromb (phlebothrombosis) and pulmonary embolism is discussed.

16. The occurrence of gangrene of the fingers or toes following cold expo in patients with cold hemagglutinins is mentioned. Of the 2 reported cases, patients had previously been working with aluminum. There is no publi account of cold hemagglutinin studies in aluminum workers.

17. In the great majority of the cases we conclude from the available infor tion that cold hemagglutination occurs in an innocuous form in many cli

conditions and may be looked upon as a laboratory curiosity. Its significance cannot be assessed at present. In some cases of hemolytic anemia, paroxysmal cold hemoglobinuria, Raynaud's syndrome, and peripheral gangrene the cold hemagglutination is of pathogenetic importance.

18. The constant presence of the phenomenon usually in low titer in normal human and animal blood is discussed. The need for restudy of its occurrence in normal human beings is pointed out.

19. There are insufficient published data to answer the query of the inheritance of cold hemagglutinins.

20. The relationship between the titer at 0°C. and the thermal amplitude of the cold hemagglutinin is discussed. In most instances the higher the titer, the broader is the amplitude.

21. The cold hemagglutinins in pathologic sera differ from those in normal sera only in that the titers and thermal amplitudes of the former are likely to be greater.

22. After proper cross-match tests, patients with cold hemagglutinins are not particularly liable to transfusion reactions.

23. A detailed description of cold agglutinin absorption is presented.

24. Cold hemagglutination results in no regularly demonstrable injury to the red blood cell. This question is discussed in detail.

25. Hemolysis in the cold in two cases of high titer cold hemagglutinins was shown to be dependent upon a high concentration of erythrocytes, extreme potency of hemagglutination and mechanical trauma. The hemolysis is fundamentally different from that of the Donath-Landsteiner test.

26. Experiments which show the paradoxical rapidity of erythrocyte sedimentation of cooled blood containing cold agglutinins are described.

27. Cold hemagglutinins have been described as globulins. By electrophoresis we have shown that cold hemagglutinins are gamma globulins. Antibody nitrogen studies revealed that a titer of cold hemagglutinins of 1/2560 was equivalent to 1.473 mg. nitrogen per cc.

28. The possibility that the individual tolerance to cold is dependent upon the titer of normal cold hemagglutinins deserves investigation.

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